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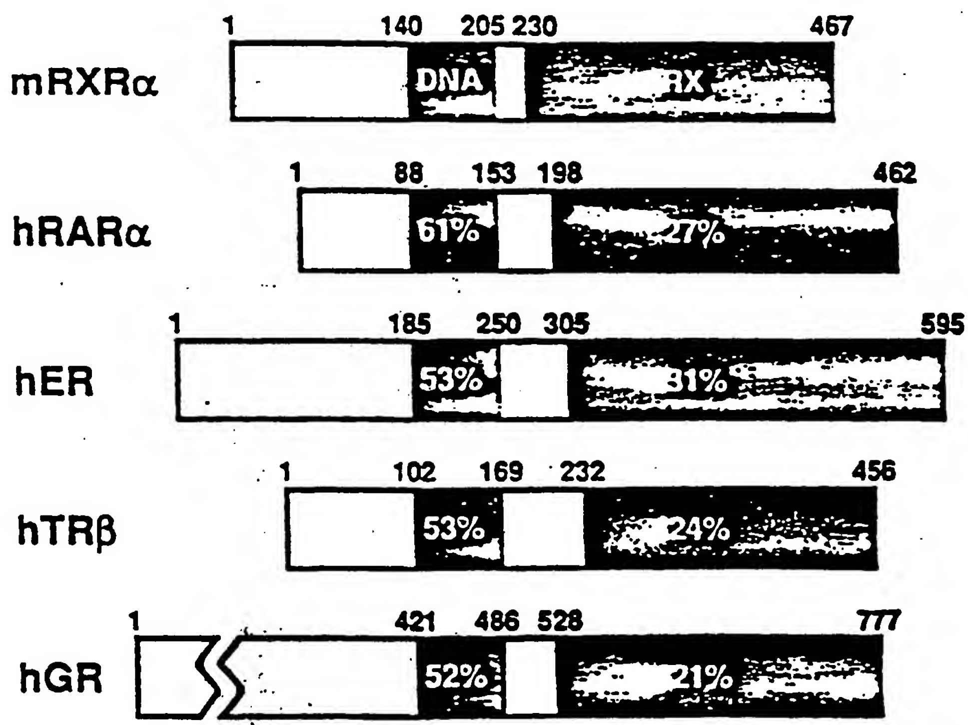
(54) Title: RETINOID RECEPTOR COMPOSITIONS AND METHODS

(57) Abstract



The present invention relates to novel receptor polypeptides, which, upon interaction with certain ligands, or activation by certain compounds, modulate transcription of certain genes by binding to cognate response elements associated with promoters of such genes. The novel receptors of the invention modulate transcription in the presence of retinoid compounds. The receptors of the present invention differ significantly from known retinoid acid receptors, in protein primary sequence and in responsiveness to exposure to various retinoids. The invention provides DNAs encoding the novel receptors, expression vectors for expression f the receptors, cells transformed with such expressi n vectors, cells co-transformed with such expressi n vectors and with reporter vectors to monitor modulation of transcription by the receptors, and methods of using such co-transformed cells in screening for compounds which are capable, directly or indirectly, f activating the receptors. The invention also provides nucleic acid probes for identifying DNAs which encode additional retinoid receptors of the same class as the novel receptors disclosed herein.

RETINOID RECEPTOR COMPOSITIONS AND METHODS

RELATED APPLICATIONS

This application is a c ntinuation-in-part of application Serial No. 478,071, filed February 9, 1990, now pending, the entire contents of which are hereby incorporated by reference herein.

TECHNICAL FIELD

The present invention concerns novel, steroid hormone-like receptor proteins and methods of making and using same.

More particularly, the invention relates to steroid hormone-like receptor proteins with transcription-modulating effects. Such proteins are responsive to th presence of retinoid acid and other vitamin A metabolites.

BACKGROUND OF THE INVENTION

The retinoids comprise a group of compounds including retinoid acid, retinol (vitamin A), and a series of natural and synthetic derivatives that togeth r exert profound effects on development and differentiation in a wide variety of systems. Although early studies focused on the effects of retinoids on growth and differentiation of epithelial cells, their actions have been shown to be widespread. Many recent studies have examined the effects of these molecules on a variety of cultured neoplastic cell types, including the human promyelocytic leukemia cell line, HL60, where retinoid acid appears to be a potent inducer of granulocyte differentiation. In F9 embryonal carcinoma cells, retinoid acid will induce the differentiation of parietal ndoderm, charact ristic of a late m us blastocyst. R tinoid acid also appears t play an important role in d fining spati -temporal axes in the developing avian limb and the regenerating amphibian limb.

Retin id acid has b n sh wn t induc th transcription f s v ral cDNAs whose gen products have b en isolated by diff rential screening. This observation supports th hypothesis that r tinoid acid exerts its action via modulation of gene expression, in a manner analogous to the way in which steroid and thyroid hormones influence their target genes.

The ability to identify compounds which affect transcription of genes which are responsive to retinoid acid or other metabolites of vitamin A, would be of significant value, e.g., for therapeutic applications. Further, systems useful for monitoring solutions, body fluids and the like for the presence of retinoid acid, vitamin A or metabolites of the latter would be of value in various analytical biochemical applications and, potentially, medical diagnosis.

Through molecular cloning studies it has been possible to demonstrate that receptors for steroid, retinoid and thyroid hormones are all structurally related. These receptors comprise a superfamily of regulatory proteins that are capable of modulating specific gene expression in response to hormone stimulation by binding directly to cis-acting elements (Evans, Science 240, 889 (1988); Green and Chambon, Trends genet. 4, 309 (1988)). Structural comparisons and functional studies with mutant receptors have established that these molecules are composed of discrete functional domains, most notably, a DNA-binding domain that is composed typically of 66 - 68 amino acids (including two zinc fingers), and an associated carboxy terminal stretch of approximately 250 amino acids which comprises the ligand-binding domain (reviewed in Evans, supra).

Low-stringency hybridization has permitted the isolation and subsequent delin ation of a gr wing list of g ne products which possess th structural f atures of hormon recept rs.

R c ntly, a r tinoid acid dependent transcripti n factor, r f rred to as RAR-alpha (r tin id acid receptor-alpha), has been identified. Subs quently, tw additi nal RAR-related genes hav been isolated; thus there are now at least thre diff rent RAR subtypes (alpha, beta and gamma) known to exist in mice and humans. These retinoid acid receptors (RARs) share homology with the superfamily of steroid hormone and thyroid hormone receptors and have been shown to regulat specific gene expression by a similar ligand-dependent mechanism (Umesono et al., Nature 336, 262 (1988)). These RAR subtypes are expressed in distinct patterns throughout development and in the mature organism.

Other information helpful in the understanding and practice of the present invention can be found in commonly assigned, co-pending United States Patent Application Serial Nos. 108,471, filed October 20, 1987; 276,536, filed November 30, 1988; 325,240, filed March 17, 1989; 370,407, filed June 22, 1989; and 438,757, filed November 16, 1989, all of which are hereby incorporated herein by reference in their entirety.

SUMMARY OF THE INVENTION

We have discovered novel receptors which are activated to modulate transcription of certain genes in animal cells, when the cells are exposed to retinoids, such as retinoid acid and retinal. The novel receptors differ significantly from known retinoid acid receptors, both in terms of the primary protein sequence and responsiveness to various retinoids.

The novel receptors have several isoforms located at genetically distinct loci. They are capable of transactivating through cis elements similar to retinoid acid receptors, but show a different rank potency and d s d pendency to retinoids. Northern analys s f the nov l receptors of th pr sent inv nti n indicat that each is form has a uniqu patt rn of expression in adult

tissue and is temporally and spatially express d in the embryo. Binding experiments demonstrat that th nov l rec ptor proteins hav a l w affinity f r [^{3}H]retinoic acid. Th se r sults, taken tog th r with r sults fr m transactivation studies, suggest the ligand(s) for the novel receptors is a metabolite(s) or structural analog(s) of retinoic acid. The invention provides DNAs encoding novel receptors, expression vectors for expression of the receptors, cells transformed with such expression vectors, cells co-transformed with such expression vectors and reporter vectors to monitor modulation of transcription by the receptors, and methods of using such co-transformed cells in screening for compounds which are capable, directly or indirectly, of activating the receptors.

The invention also provides single-stranded nucleic acid probes for identifying DNAs encoding additional retinoid receptors.

The invention also provides a method for making th receptors of the invention by expressing DNAs which encode the receptors in suitable host organisms.

Animal cells in which receptors of the invention are present can be employed to assay fluids for the presenc of retinoids. Animal cells of the invention can also be employed to screen compounds of potential therapeutic value for their ability to bind and/or promote trans-activation (i.e., trans-acting transcriptional activation) by the receptors of the invention.

As will be described in greater detail below, the receptors of the invention modulate transcription of genes. This occurs upon binding of receptor to hormone response elements, which are positioned operatively, with respect to promoters for such genes, for such modulation t occur. Am ng horm ne r sp ns lements cont mplated for use in the practice of th pres nt inventi n ar TRE_p, the b ta-retin id acid r spons el m nt, and th estrogen r spons lem nt, as well as closely r lated lements

which ar disclosed, for example, in Application Serial No. 438,757, filed N vember 16, 1989, and Applicati n Serial No. 325,240, filed March 17, 1989.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the extent of amino acid identity (i.e., "homology") between the DNA binding domain ("DNA") and ligand binding domain ("RX") of mouse RXR-alpha (mRXRα), relative to the corresponding domains of human retinoic acid receptor-alpha (hRARα), human estrogen receptor (hER), human thyroid hormone receptor-beta (hTRβ) and human glucocorticoid receptor (hGR).

Figure 2 shows the extent of amino acid identity (i.e., "homology") between the DNA binding domain ("DNA") and ligand binding domain ("LIGAND") of human RXR-alpha (hRARα), relative to the corresponding domains of human retinoic acid receptor-beta (hRARβ), human retinoic acid receptor-gamma (hRARγ), hTRβ and hRXRα.

Figure 3 shows the extent of amino acid identity (i.e., "homology") between the DNA binding domain ("DNA") and ligand binding domain ("RX") of mRXRα, relative to the corresponding domains of mouse RXR-beta (mRXRβ), mouse RXR-gamma (mRXRγ) and hRXRα.

Figure 4 illustrates the production of CAT from the reporter vector (ADH-TREp-CAT) in Drosophila melanogaster Schneider line 2 cells, which are co-transformed with receptor expression vector A5C-RXR-alpha and are in a medium containing various concentrations of retinoic acid.

Figure 5 illustrates the differences in transcription-activating activities of hRXR-alpha and hRAR-alpha, in mammalian cells in culture containing different vitamin A metabolites.

Figure 6, like Figure 5, illustrates the diff r nc s in transcription-activating activiti s f hRXR-alpha and hRAR-alpha in mammalian c lls in culture c ntaining r tin ic acid or different synth tic r tinoids.

Figure 7 illustrates the differ nces b tw n hRXR-alpha and hRAR-alpha in dos -respons to r tinoic acid in media bathing mammalian c lls in which th r cept rs occur. Figure 8 illustrat s th differenc s b tw en mouse RXR-alpha (mRXRα), mouse RXR-beta (mRXRβ) and mous RXR-gamma (mRXRγ) in dose response to retinoid acid (RA) in media bathing mammalian cells expressing such receptors.

Figure 9 illustrates the differences between mRXRα, mRXRβ and mRXRγ in dose response to 3,4-didehydroretinoic acid (ddRA) in media bathing mammalian cells expressing such receptors.

DETAILED DESCRIPTION OF THE INVENTION

The invention concerns novel polypeptides, which ar characterized by:

(1) being responsive to the presence of retinoid(s) to regulate transcription of associated gene(s);

(2) having a DNA binding domain of about 66 amino acids with 9 Cys residues, wherein said DNA binding domain has:

(a) less than about 65 % amino acid identity with the DNA binding domain of hRAR-alpha,

(b) less than about 55 % amino acid identity with the DNA binding domain of hTR-beta, and

(c) less than about 55 % amino acid identity with the DNA binding domain of hGR; and

(3) not including the sequence set forth in Sequence ID No 7.

The novel polypeptide receptors of the present invention can be further charact riz d in a variety of ways, e.g., by increasing the rate f transcription of a targ t g n in a construct comprising a promoter operatively link d t a h rm n r spons el ment f r

transcriptional activation by said r c ptors, relativ to th rate f transcription in th abs nce of said r c pt r and/or in th absence of r tinoic acid and retinal. Transcription of said targ t g n is measur d in an animal cell in cultur , the medium f which compris s retinoid acid or retinal at a concentration greater than about 5 x 10^{-7} M.

Alternatively, the polypeptide receptors of the present invention can be further characterized as being encoded by a continuous nucleotide sequence which encodes substantially the same amino acid sequence as that of amino acids 1-462 shown in Sequence ID No. 2 [hRXRα], amino acids 1-467 shown in Sequence ID No. 4 [mRXRα], or amino acids 1-463 shown in Sequence ID No. 6 [mRXRγ].

As yet another alternative, the polypeptide receptors of the present invention can be characterized as being encoded by a continuous nucleotide sequence which encodes substantially the same amino acid sequenc as that of amino acids 135-200 shown in Sequence ID No. 2 [DNA binding domain of hRXRα], amino acids 140-205 shown in Sequence ID No. 4 [DNA binding domain of mRXRα], or amino acids 139-204 shown in Sequence ID No. 6 [DNA binding domain of mRXRγ].

As still another alternative, the polypeptide receptor of the present invention can be characterized as being encoded by a continuous nucleotide sequence which is substantially the same as nucleotides 76-1464 shown in Sequence ID No. 1 [hRXRα], nucleotides 181-1581 shown in Sequence ID No. 3 [mRXRα], or nucleotides 123-1514 shown in Sequence ID No. 3 [mRXRγ].

As employed herein, the term "retinoids" refers to naturally occurring compounds with vitamin A activity synthetic analogs and various metabolites thereof. The retinoids are a class of compounds consisting of four isopren id units joined in h ad-t -tail manner. Numer us r tinoids hav been id ntifi d, as d scrib d, for xampl , by Sporn, Roberts and G odman in

th tw volum treatise entitled The Retinoids (Academic Press, NY, 1984), to which the r ad r is dir cted f r furth r d tail. Exemplary retinoids includ retin l, retinyl acetate, retinyl hexadecanoate, α-r tinyl, 4,14-retroretinol, deoxyretinol, anhydroretinol, 3,4-didehydroretinol, 15,15-dimethyl retinol, retinyl methyl ether, retinyl phosphate, mannosyl retinyl phosphate, retinol thioacetate, retinal (retinaldehyde), 3,4-didehydroretinal, retinylidene acetylacetone, retinylidene-1,3-cyclopentanedione, retinal oxime, retinaldehyde acetylhydrazone, retinoic acid, 4-hydroxyretinoic acid, 4-oxoretinoic acid, 5,6-dihydroretinoic acid, 5,6-epoxyretinoic acid, 5,8-epoxyretinoic acid, the open-chain C_{20} analog of retinoid acid (i.e., (all-E-3,7,11,15-tetramethyl-2,4,6, 8,10, 2,14-hexadecaheptaenoic acid), 7,8-didehydroretinoic acid, 7,8-dihydroretinoic acid, "C_{15} Acid" (E, E)-3-methyl-5-(2,6,6-trimethyl-2-cyclohexen-1-yl)-2,4-pentanedioic acid), "C_{17} Acid" ((E,E,E)-5-methyl-7-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6-hepatrienoic acid), "C_{22} Acid" (14'-apo-β, ψ-carotenoic acid), retinoic acid esters (e.g., methyl ester, ethyl ester, etc.), retinoid acid ethylamide, retinoic acid 2-hydroxyethylamide, methyl retinone, "C_{18}" Ketone" ((E,E, E)-6-methyl-8-(2,6,6-trimethyl-1-cyclohexen-1-yl)-3,5,7-ocatrien-2-one), and the like.

In addition, according to the present invention, there are provided DNA sequences which encode novel polypeptides as described above.

Further in accordance with the present invention, there are provided DNA constructs which are operative in animal cells in culture to make said polypeptides.

According to a still further embodiment of the present invention, there are provided animal cells in cultur which ar transf rm d with DNA constructs (as d scrib d abov), which ar op rativ in said cells to

mak receptor polypeptides, by expr ssi n of DNA segments which encode the above described polypeptides.

Among th animal cells contemplated for us in the practice of th pres nt invention are those which are furth r transformed with a reporter v ct r which comprises:

(a) a promoter that is operable in the cell,

(b) a hormone response element, and

(c) a DNA segment encoding a reporter protein,

wherein said reporter protein-encoding DNA segment is operatively linked to said promoter for transcription of said DNA segment, and

wherein said hormone response element is operatively linked to said promoter for activation thereof.

In accordance with the present invention, there is also provided a method of testing a compound for its ability to regulate the transcription-activating properties of the above-described receptor polypeptides, which method comprises assaying for the presence or absence of reporter protein upon contacting of cells containing a reporter vector and receptor polypeptide with said compound; wherein said reporter vector and said receptor polypeptide are as described above.

In accordance with a still further embodiment of th present invention, there are provided various probes, which can be used to identify genes encoding receptors related to those of the present invention. In this regard, particular reference is made to Examples V and VI below. More particularly, the invention provides labeled, single-stranded nucleic acids comprising sequences of at least 20 contiguous bases having substantially the same sequence as any 20 or more contiguous bases selected from:

(i) bas s 2 - 1861, inclusiv , of th DNA illustrat d in Sequenc ID No. 1 [hRXR-α], r

(ii) bas s 20 - 2095, inclusive, of the DNA illustrated in S quenc ID No. 2 [mRXR-α], or

(iii) bases 15 - 1653, inclusiv , f the DNA illustrated in Sequence ID No. 3 [mRXR-γ], or

(iv) the complement of any one of the sequences according to (i), (ii), or (iii).

As employed herein, the term "labeled single-stranded nucleic acid sequences" refers to single-stranded DNA or RNA sequences which have been modified by the addition thereto of a species which renders the "labeled" sequence readily detectable from among other unmodified sequences. Exemplary labels include radioactive label (e.g., ^{32}P, ^{35}S), enzymatic label (e.g., biotin), and the like.

Preferred probes contemplated for use in the practice of the present invention are those having at least about 100 contiguous bases selected from the above-described sequences. Especially preferred are probes having in the range of about 198 up to several hundred nucleotides, because greater selectivity is afforded by longer sequences.

The invention also encompasses a method of making the above-described receptor polypeptides, which method comprises culturing suitable host cells which are transformed with an expression vector operable in said cells to express DNA which encodes receptor polypeptide. Suitable hosts contemplated for use in the practice of the present invention include yeast, bacteria, mammalian cells, insect cells, and the like. E. coli is the presently preferred bacterial species. Any of a number of expression vectors are well known to those skilled in th art that c uld b mployed in th method f th inventi n. Among th se ar th prokaryotic expr ssi n v ct rs pNH8A, pNH16A and pNH18A availabl from Stratagene, La Jolla, Calif rnia USA.

Further information on the invention is provided in the f ll wing non-limiting xamples and descripti n f an xemplary d posit.

EXAMPLES

Example I

The KpnI/SacI restriction fragment (503bp) including the DNA-binding domain of hRAR-alpha-encoding DNA [See Giguere et al., Nature 330, 624 (1987); and commonly assigned United States Patent Application Serial No. 276,536, filed November 30, 1988; and European Patent Application Publication No. 0 325 849, all incorporated herein by reference] was nick-translated and used to screen a lambda-gt11 human liver cDNA library (Kwok et al.,Biochem. 24, 556 (1985)) at low stringency. The hybridization mixture contained 35% formamide, 1X Denhardt's, 5X SSPE (1X SSPE=0.15 M NaCl, 10mM Na_2HPO_4 1mM EDTA), 0.1% SDS, 10% dextran sulfate, 100 mg/ml denatur d salmon sperm DNA and 10^6 cpm of [^{32}P]-labelled probe. Duplicate nitrocellulose filters were hybridized for 16h at 42°C, washed once at 25°C for 15 min with 2X SSC (1X SSC=0.15 M NaCl, 0.015 M sodium citrate), 0.1% SDS and then washed twice at 55°C for 30 min. in 2X SSC, 0.1% SDS. The filters were autoradiographed for 3 days at -70°C using an intensifying screen.

Positive clones were isolated, subcloned into pGEM vectors (Promega, Madison, Wisconsin, USA), restriction mapped, and re-subcloned in various sized restriction fragments into M13mp18 and M13mp19 sequencing vectors. DNA sequence was determined by the dideoxy method with Sequenase™ sequencing kit (United States Biochemical, Cleveland, Ohio, USA) and analyzed by University of Wisconsin Genetics Computer Group programs (Devereux et al., Nucl. Acids Res. 12, 387 (1984)). A unique receptor-like sequence was identifi d and designated lambda-HL3-1.

Lambda-HL3-1 was us d as a hybridization probe to rescr n a lambda-gt10 human kidney cDNA library (Arriza et al., Science 237, 268 (1987)) which produc d several clones, the long st of which was s quenc d and designat d lambda-XR3-1. The DNA sequence obtained as an EcoRI-fragment from lambda-XR3-1 has the sequence indicated in Sequence ID No. 1 [hRXRα].

Similar screening of a mouse whole embryo library with the full-length hRXR-alpha clone described above provided additional sequences which encode different isoforms of the human RXR-alpha receptor. In addition, the mouse homolog (mouse RXR-alpha) was also identified in this way.

Thus, mRNA was isolated from 14.5 day post-coitus (p.c.) mouse embryos, translated into cDNA, linkered with EcoRI/NotI linkers, then inserted into the unique EcoRI site of the cloning vector λ-ZAP (Stratogene). The resulting library was screened at reduced stringency with ^{32}P-labeled, full length hRXR-alpha as the probe.

The DNA sequences of the resulting clones are set forth as Sequence ID No. 3 [mRXRα] and Sequence ID No. 5 [mRXRγ].

Example II

Amino acid sequences of mRXR-alpha, hRAR-alpha (human retinoic acid receptor-alpha), hER (human estrogen receptor) hTR-beta (human thyroid hormone receptor-beta) and hGR (human glucocorticoid receptor) were aligned using the University of Wisconsin Genetics Computer Group program "Bestfit" (Devereux et al., supra). Regions of significant similarity between mRXR-alpha and the other receptors, i.e., the 66 - 68 amino acid DNA binding domains and the ligand-binding domains, are presented schematically in Figur 1 as perc nt amino acid id ntity.

Similarly, th amino acid s qu nces of human RAR-alpha (hRARα), human RAR-beta (hRARβ), human RAR-gamma (hRARγ), human TR-b ta (hTRβ) and human RXR-alpha (hRXRα)

were align d. As done in Figur 1, regions f significant similarity betw en hRAR-alpha and the oth r r c pt rs are pr s nt d schematically in Figur 2 as percent amin acid identity.

A furth r comparison of r ceptors is set forth in Figure 3. Thus, the amino acid sequences of mouse RXR-alpha (mRXRα), mouse RXR-beta (mRXRβ), mouse RXR-gamma (mRXRγ) and human RXR-alpha (hRXRα) were aligned, and th percent amino acid identity presented schematically in Figure 3.

Although the DNA-binding domains of both mRXR-alpha and hRXR-alpha are conserved relatively well with respect to other receptors (such as hRAR-alpha and hTR-beta), the ligand binding domain is poorly conserved. (See Figures 1 and 3). A comparison between the retinoic acid receptor subfamily of receptors and hRXR-alpha reveals nothing to suggest that hRXR-alpha is related to any of the known retinoid receptors (Fig. 2).

Example III

Drosophila melanogaster Schneider line 2 ("S2") cells (Schneider, Embryol. Exp. Morphol. 27, 353 (1972), which are readily available, were seeded at 2 x 10^6 per 35 mm^2 culture dish and maintained in Schneider medium (GIBCO/Life Technologies, Inc., Grand Island, New York, USA) supplemented with penicillin, streptomycin and 12% heat-inactivated fetal bovine serum (Irvine Scientific, Santa Ana, California, USA). The cells were transiently co-transfected with 10 μg/dish of plasmid DNA by calcium phosphate precipitation (Krasnow et al., Cell 57, 1031 (1989): 4.5 μg/dish of receptor expression vector or control construct (producing no hRXR-alpha); 0.5 μg/dish of reporter plasmid or control reporter plasmid; 0.5 μg/dish of reference plasmid; and 4.5 μg inert plasmid DNA.

In th r c pt r xpression v ct r, A5C-RXR-alpha (4.5 μg/dish), r c ptor hRXR-alpha is constitutively

expr ss d in the S2 cells under the control of the Drosophila actin 5C promoter (A5C; Thummel t al., Gene 74: 445 (1988)) driving transcription of the EcoRI-site-bound d ins rt of lambda-XR3-1. In th control vector, A5C-RXR_{rev} (also 4.5 µg/ml), the EcoRI-site-bounded insert from lambda-XR3-1 is inserted in the reverse (i.e., non-coding or non-sense-coding) orientation.

A5C-RXR-alpha was made by first inserting at the unique BamHI site of A5C a linker of sequence:

5'-GATCCGATATCCATATGGAATTCGGTACCA,

and then inserting, at the EcoRI site of the linker (underlined above), the EcoRI-site-bounded insert of lambda-XR3-1 (See Example I).

The reporter plasmid ADH-TRE_p-CAT (at 0.5 µg/dish) contains the palindromic thyroid hormone response element TREp, having the sequence:

5'-AGGTCATGACCT

[(Glass et al. Cell 54, 313 (1988); Thompson and Evans, Proc. Natl. Acad. Sci. (USA) 86, 3494 (1989)], inserted into position -33 (with respect to the transcription start site) of a pD33-ADH-CAT background (Krasnow et al., Cell 57, 1031 (1989)).

pD33-ADH-CAT is a plasmid with the distal promoter of the Drosophila melanogaster alcohol dehydrogenase gene linked operably for transcription to the bacterial (E. coli) chloramphenicol acetyltransferase ("CAT") gene, a gene for the indicator protein CAT. ADH-TREp-CAT was made by inserting the oligonucleotide of sequence:

```
5'-CTAGAGGTCATGACCT
        TCCAGTACTGGAGATC-5'
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into the XbaI sit at positi n -33 in pD33-ADH-CAT. pD33-ADH-CAT, without TREp, served as a control reporter (i.e., background) plasmid.

A r fer nce plasmid encoding beta-galactosidas driven by the actin 5C promoter also was transfected (0.5 μg/dish) along with pGEM DNA (4.5 μg/dish) (Promega, Madison, Wisconsin) to make up the final DNA concentration to 10 μg/dish. The reference plasmid was made by inserting a BamHI-site bounded, beta-galactosidase-encoding segment into the unique BamHI site of A5C. The purpose of the reference plasmid was to normalize results for transfection efficiency.

Twenty-four hours post-transfection, various retinoids were added to the cultures. The retinoids were dissolved in dimethyl-sulfoxide and/or ethanol and the resulting solution was added to 0.1 % v/v of culture medium. Initial concentration of the retinoids in the culture media was 10^{-6} M, except for the experiments underlying the data displayed in Figure 4, for which varying concentrations of retinoic acid were used.

In control runs, ethanol, at 0.1 % v/v in the medium, was used in place of a solution of retinoid.

Cultures were maintained in the dark for 36 hr after addition of retinoid and then harvested. All other parts of the experiments, involving retinoids, were carried out in subdued light.

Cell lysates were centrifuged. Supernatants were assayed for beta-galactosidase, following Herbomel et al., Cell 39, 653-662 (1984), and units/ml of beta-galactosidase activity was calculated. CAT assays (normalized to beta-galactosidase activity) of supernatants were incubated for 75 unit-hours ("units" referring to units of beta-galactosidase activity), as described by Gorman et al., Mol. Cell. Biol. 2, 1044 (1982), usually 150 units f r 30 minutes.

No hRXR-alpha dep nd nt activation of CAT xpr ssion was noted in any exp rim nt in which control reporter was

used in place of ADH-TREp-CAT. Similarly, essentially no activation was observed for runs wher control plasmid, A5C-$hRXR_{rev}$, was us d in plac of A5C-hRXR.

The induction of CAT activity in retinoid-treat d cells was compared with induction in untreated (i.e., only ethanol-treated) cells. Induction was measured in the presence of retinoic acid (RA), retinal (RAL), retinol acetate (RAC), retinol (ROH), and retinol palmitate (RP). The production of chloramphenicol acetyltransferase (CAT) from the reporter vector (ADH-TREp-CAT) was measured in Drosophila melanogaster Schneider line 2 cells, co-transformed with the hRXR-alpha expression vector A5C-RXR-alpha, and exposed to a medium to which retinoic acid (RA), retinal (RAL), retinol acetate (RAC), retinol (ROH), or retinol palmitate (RP) has been added to a concentration of 10^{-6} M. The relative induction observed was RA > RAL > RAC > ROH > RH.

In Figure 4 are displayed the results, also expressed in terms of "fold-induction" of CAT activity, as described in the previous paragraph, with retinoic acid at a number of different concentrations, to show the "dose response" of hRXR-alpha (in trans-activation at TREp in insect cells) to retinoid acid in the medium of the cells.

Example IV

This example, describing experiments similar to those described in Example III, shows that hRAR-alpha and hRXR-alpha differ significantly in their properties, specifically with respect to trans-activation of transcription from promoters.

The mammalian receptor-expression vector RS-hRAR-alpha, from which hRAR-alpha is produced under control of the 5'-LTR promoter of the rous sarcoma virus proviral DNA, is described in Giguere et al., Natur 330, 624 (1987); c mm nly asign d United Stat s Pat nt Application Serial No. 276,536, filed November 30, 1988; and European

Patent Application Publication No. 0 325 849, all incorporated herein by reference.

Th rec ptor-expression vector RS-hRXR-alpha is constructed similarly to RS-hRAR-alpha, by inserting the EcoRI-site-bounded, hRXR-alpha-encoding segment of lambda-XR3-1 into plasmid pRS (Giguere et al., Cell 46, 645 (1986)).

Control plasmid pRSns is pRS with a non-sense-coding sequence inserted in place of receptor-coding sequence.

Reporter plasmid delta-MTV-TREp-CAT, also known as TREp1MCAT, has also been described (Umesono et al., Nature 336, 262 (1988), Thompson and Evans, supra., see also Umesono and Evans, Cell 57, 1139 (1989). When a control reporter, designated delta-MTV-CAT, which is substantially delta-MTV-TREp-CAT with TREp removed, was used in place of delta-MTV-TREp-CAT, no CAT activity was found with either receptor with any of the retinoids or retinoid analogs.

Reference plasmid, RS-beta-galactosidase, is also known and is substantially the same as RS-hRAR-alpha and RS-hRXR-alpha but has a beta-galactosidase-encoding segment in place of the receptor-encoding segment.

Culture of CV-1 cells, co-transfections (with reporter plasmid, receptor-expression-plasmid or control plasmid, reference plasmid and inert plasmid DNA) and CAT assays were performed as described in Umesono et al., Nature 336, 262 (1988). Co-transfections and CAT assays were carried out by methods similar to those described in Example III. Similar to the experiments in Example III, subdued light was used.

When CV-1 cells co-transformed with reporter plasmid (delta-MTV-TREp-CAT), reference plasmid, control plasmid (i.e., expressing no receptor), and receptor plasmid (RS-hRAR-alpha or RS-hRXR-alpha), were exposed to retinoids RA, RAL, RAC, ROH, RP, (which are naturally occurring vitamin A m tabolit s), or r tinoid-fr ethanol, th results shown in Figur 5 w r obtain d. The Figur illustrates production f CAT from reporter plasmid

in monkey kidney cells f the CV-1 line, which are co-transformed with hRXR-alpha-producing expression v ctor RS-hRXR-alpha or hRAR-alpha-producing expr ssion vector RS-hRAR. Experiments are carri d out in a m dium to which RA, RAL, RAC, ROH, or RP has been added to a concentration of 10^{-6} M. The bars over the "-" sign indicate the levels of CAT production when the cells are exposed to no retinoid (i.e., retinoid-free ethanol). The hatched bars indicate the level of CAT production when a control expression vector, from which no receptor is expressed, is employed in place of the receptor expression vector. The open bars indicate the level of CAT production when receptor-producing expression vector is employed. In each case, the retinoids were added as ethanolic solutions, with the volume of solution 0.1 % (v/v) in the medium. Retinoid-free ethanol was added to 0.1 % v/v. Results are plotted as percentages of the maximal response observed in the experiments, i.e., hRXR-alpha with RA.

In Figure 6, the results are provided for experiments carried out as described in the previous paragraph but with, in place of RAL, RAC, ROH and RP, the synthetic retinoids 4-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-4-iodo-2-antrhracenyl)-benzoic acid ("R1"), ethyl-P-[(E)-2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthyl)-1-propenyl]-benzoic acid ("R2"), ethyl-all trans-9-(4-methoxy-2,3,6-trimethyl)-3,7-dimethyl-2,4,6,8-nonatetranoate ("R3"), and ethyl-all trans-9-(4-methoxy-2,3,6-trimethyl)-3,7-dimethyl-2,4,6,8-nonatetraenoic acid ("R4") initially at a concentration of 10^{-6} M. The Figure illustrates production of CAT from the reporter plasmid (delta-MTV-TREp-CAT), CV-1 cells, which are co-transformed with hRXR-alpha-producing expression vector RS-hRXR-alpha or the constitutive hRAR-alpha-producing expression vector RS-hRAR. Experiments are carried out in a medium to which RA, R1, R2, R3, or R4 has b en added to a c ncentration f 10^{-6} M. Th bars over the "-" sign indicate the l vels f CAT production when the c lls are exposed to no retinoid.

The hatched bars indicate th level f CAT production when a control expression vector, from which no rec ptor is express d, is employ d in plac of the r c ptor expression vector. The open bars indicat the level of CAT production when r ceptor-producing expression v ctor is employed.

In Figure 7, results are presented for experiments carried out as described in this Example using various concentrations of retinoic acid. The Figure illustrates production of CAT from the reporter plasmid (delta-MTV-TRE_p-CAT), in CV-1 cells, which are co-transformed with the receptor-producing expression vector RS-RXR-alpha or RS-RAR-alpha. Experiments are carried out in a medium to which RA has been added to various concentrations. In the Figure, the results are in terms of fold-induction observed with cells exposed to RA, and control cells (exposed to only RA-free ethanol).

In Figure 8, results are presented for experiments carried out as described above, using various concentrations of retinoic acid with expression vectors encoding mRXR-alpha, mRXR-beta and mRXR-gamma.

In Figure 9, results are presented for experiments carried out as described above, using various concentrations of 3, 4-didehydroretinoic acid (ddRA) with expression vectors encoding mRXR-alpha, mRXR-beta and mRXR-gamma.

Example V

To determine the distribution of hRXR-alpha gene expression, poly A^+ RNAs isolated from a variety of adult rat tissues were size fractionated, transferred to a nylon filter, and hybridized with hRXR-alpha cDNA.

Thus, for each tissue of adult male rat that was analyzed, total RNA was prepared from the tissue (see Chomczynski and Sacchi, Anal. Bioch m. 162, 156 (1987)) and poly A^+ s l cted by oligo(dT)-c llulos chromatography. T n micrograms of poly A^+ RNA were separated by 1% agarose-

formaldehyd g l lectrophoresis, transf rred to a Nytran filter (Schleicher and Schuell) (see McDonnell et al., Sci nc 235, 1214 (1987)), and hybridized under string nt conditions with the hRXR-alpha-encoding, EcoRI insert of lambda-XR3-1. Hybridization was performed at 42°C in a buffer containing 50% formamide, 5X Denhardt's, 5X SSPE, 0.1% SDS, 100mg/ml salmon sperm DNA, 200mg/ml yeast RNA, and [^{32}P]-labelled probe. The filter was then washed twice with 2X SSC, 0.1% SDS at 22°C and twice at 50°C. Autoradiography was for 24h at -70°C with an intensifying screen. RNA ladder size markers from Bethesda Research Laboratories (Gaithersburg, Maryland, USA)

The distribution of RXR-alpha mRNA in the rat reveals a pattern of expression distinct from that of the retinoid acid receptors (Giguere et al., Nature 330, 624 (1987); Zelent et al., Nature 339, 714 (1989); Benbrook, Nature 333, 669 (1988)). The rat RXR-alpha message appears to be a single species of about 4.8 kbp (kilobase pairs) which is expressed in many tissues, but most abundantly in the liver, muscle, lung, and kidney and somewhat less abundantly in adrenal, heart, intestine, and spleen.

Example VI

Molecular cloning analyses of the thyroid hormone and retinoic acid receptor genes indicate that each of these receptors belongs to a discreet gene subfamily which encode several receptor isoforms. To determine if this was also true of RXR, a series of Southern blot analyses were carried out. High stringency hybridization of restriction endonuclease-digested human DNA with a labelled DNA fragment derived from lambda-XR3-1 produced a similar number of bands in every digestion, consistent with a single genetic locus. When the hybridization conditi ns were r lax d, howev r, many additional bands w r bs rv d in th products of each nzyme digestion. Car ful inspecti n of this hybridizati n pattern demonstrat d that it is unrelat d to a similar analysis

described for hRAR-alpha (Giguere et al., Nature 330, 624 (1987). These observations indicate the presence of at least one other locus in th human g nome related to the hRXR-alpha g n . Further, a genomic DNA zooblot representing mammalian, avian, yeast, and Drosophila species was obtained. Thus far, the RXR gene family appears to be present in all species tested except yeast, which to date has not been shown to contain any members of the steroid receptor superfamily.

For the analyses of human DNA, two human placenta genomic DNA Southern blots were prepared in parallel with identical DNA samples. The blots were hybridized at high or low stringency with a ~1200 bp [^{32}P]-labelled fragment of lambda-XR3-1 which included the coding portions of the DNA and ligand binding domains (Sequence ID No. 1, nucleotides 459-1631).

For the zooblot, genomic DNA from human, monkey, rat, mouse, dog, cow, rabbit, chicken, S. cerevisiae and Drosophila melanogaster were hybridized at low stringency with a ~330 bp [^{32}P]-labelled fragment of lambda-XR3-1 which included the DNA-binding domain (Sequence ID No. 1, nucleotides 459-776). Differently sized bands (in comparison with HindIII-digested lambda DNA for sizing) were found for the various species. The blots for all of the species (including both for D. melanogaster), except yeast, mouse and rabbit appeared to have more than one band.

For the analysis of human DNA, the placental DNA was restricted with BamHI, BglII, EcoRI, HindIII, PstI and PvuII, separated in a 0.8% agarose gel (10 μg per lane) and transferred to nitrocellulose (see McDonnell et al., supra) and hybridized as described below.

For the zooblot, EcoRI-digested DNA from the several species (Clontech, Palo Alto, California, USA), other than D. melanogaster, was used for Southern blot analysis. EcoRI- and XhoI-dig sted D. melanogast r DNA was included also.

Bl ts w re hybridized at 42°C in the low stringency buffer described in Example I or at high stringency in th same buffer modified by addition of formamide to 50 %. Low stringency blots wer wash d twic at room temperatur and twice at 50°C in 2X SSC, 0.1% SDS. The high stringency blot was washed twice at room temperature in 2X SSC, 0.1% SDS and twice at 65°C in 0.5X SSC, 0.1% SDS.

Example VII

Northern analysis were carried out on the mouse RXR isoforms alpha, beta and gamma, to determine the tissue distribution of these receptors in adult tissues and in developing embryos.

Thus, mRNA (10μg) was isolated from various adult rat tissues of from day 10.5-day 18.5 p.c. whole mouse embryos. These samples were subjected to Northern analysis using ^{32}P-labeled cDNA probes derived from regions specific to mRXRα, mRXRβ, or mRXRγ.

In the adult, the various RXR isoforms are seen to be expressed in both a specific and overlapping distribution pattern.

In the embryo, the various isoforms are highly expressed in what appears to be a specific temporal pattern.

The invention has been described in detail with particular reference to preferred embodiments thereof, but it will be understood that variations and modifications can be effected within the spirit and scope of the invention.

Deposit

On January 31, 1990, a sample of replicatable phagescript SK doubl -stranded DNA (Stratagene, La Jolla, California, USA), with the 1860 base-pair, EcoRI-site-bounded DNA, the sequence of which is illustrated in Figure 1, inserted at the unique EcoRI site, was deposited under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure at the American Type Culture Collection, Rockville, Maryland, USA ("ATCC"). The accession number assigned to this deposit is ATCC 40741. The deposited DNA is designated pSK(hRXR-alpha).

Phagescript SK double-stranded DNA is a modified M13mp18 bacteriophage DNA (double-stranded). Derivatives, such as pSK(hRXR-alpha), of phagescript SK double-stranded DNA can be cloned in the same way as M13mp18 and its derivatives.

Samples of pSK(hRXR-alpha) will be publicly available from the ATCC without restriction, except as provided in 37 CFR 1.801 et seq., at the latest on the date an United States Patent first issues on this application or a continuing application thereof. Otherwise, in accordance with the Budapest Treaty and the regulations promulgated thereunder, samples will be available from the ATCC to all persons legally entitled to receive them under the law and regulations of any country or international organization in which an application, claiming priority of this application, is filed or in which a patent based on any such application is granted.

SUMMARY OF SEQUENCES

Sequence ID No. 1 is th coding sequence of an EcoRI-site-bounded DNA s gment which enc des the novel receptor disclosed her in, referred to as human RXR-alpha [hRXRα]

Sequence ID No. 2 is the amino acid sequence of the novel receptor referred to herein as hRXRα.

Sequence ID No. 3 is the nucleotide (and amino acid) sequence of the novel receptor disclosed herein, referred to as mouse RXR-alpha [mRXRα].

Sequence ID No. 4 is the amino acid sequence of the novel receptor referred to herein as mRXRα.

Sequence ID No. 5 is the nucleotide (and amino acid) sequence of the novel receptor disclosed herein, referred to as mouse RXR-gamma [mRXRγ].

Sequence ID No. 6 is the amino acid sequenase of the novel receptor referred to herein as mRXRγ.

Sequence ID No. 7 is the nucleotide sequence of the receptor disclosed by Hamada, et al in PNAS 86: 8298-8293 (1989). This receptor is similar to the receptor referred to herein as mRXRβ.

SEQ ID NO:1:

```
GAATTCCGGC GCCGGGGGCC GCCCGCCCGC CGCCCGCTGC CTGCGCCGCC GGCCGGGCAT        60

GAGTTAGTCG CAGAC ATG GAC ACC AAA CAT TTC CTG CCG CTC GAT TTC TCC        111
                 Met Asp Thr Lys His Phe Leu Pro Leu Asp Phe Ser
                  1               5                  10

ACC CAG GTG AAC TCC TCC CTC ACC TCC CCG ACG GGG CGA GGC TCC ATG         159
Thr Gln Val Asn Ser Ser Leu Thr Ser Pro Thr Gly Arg Gly Ser Met
         15                  20                  25

GCT GCC CCC TCG CTG CAC CCG TCC CTG GGG CCT GGC ATC GGC TCC CCG         207
Ala Ala Pro Ser Leu His Pro Ser Leu Gly Pro Gly Ile Gly Ser Pro
 30                  35                  40

GGA CAG CTG CAT TCT CCC ATC AGC ACC CTG AGC TCC CCC ATC AAC GGC         255
Gly Gln Leu His Ser Pro Ile Ser Thr Leu Ser Ser Pro Ile Asn Gly
 45                  50                  55                  60

ATG GGC CCG CCT TTC TCG GTC ATC AGC TCC CCC ATG GGC CCC CAC TCC         303
Met Gly Pro Pro Phe Ser Val Ile Ser Ser Pro Met Gly Pro His Ser
                 65                  70                  75

ATG TCG GTG CCC ACC ACA CCC ACC CTG GGC TTC AGC ACT GGC AGC CCC         351
Met Ser Val Pro Thr Thr Pro Thr Leu Gly Phe Ser Thr Gly Ser Pro
             80                  85                  90

CAG CTC AGC TCA CCT ATG AAC CCC GTC AGC AGC AGC GAG GAC ATC AAG         399
Gln Leu Ser Ser Pro Met Asn Pro Val Ser Ser Ser Glu Asp Ile Lys
         95                 100                 105

CCC CCC CTG GGC CTC AAT GGC GTC CTC AAG GTC CCC GCC CAC CCC TCA         447
Pro Pro Leu Gly Leu Asn Gly Val Leu Lys Val Pro Ala His Pro Ser
    110                 115                 120

GGA AAC ATG GCT TCC TTC ACC AAG CAC ATC TGC GCC ATC TGC GGG GAC         495
Gly Asn Met Ala Ser Phe Thr Lys His Ile Cys Ala Ile Cys Gly Asp
125                 130                 135                 140

CGC TCC TCA GGC AAG CAC TAT GGA GTG TAC AGC TGC GAG GGG TGC AAG         543
Arg Ser Ser Gly Lys His Tyr Gly Val Tyr Ser Cys Glu Gly Cys Lys
                145                 150                 155

GGC TTC TTC AAG CGG ACG GTG CGC AAG GAC CTG ACC TAC ACC TGC CGC         591
Gly Phe Phe Lys Arg Thr Val Arg Lys Asp Leu Thr Tyr Thr Cys Arg
            160                 165                 170

GAC AAC AAG GAC TGC CTG ATT GAC AAG CGG CAG CGG AAC CGG TGC CAG         639
Asp Asn Lys Asp Cys Leu Ile Asp Lys Arg Gln Arg Asn Arg Cys Gln
        175                 180                 185

TAC TGC CGC TAC CAG AAG TGC CTG GCC ATG GGC ATG AAG CGG GAA GCC         687
Tyr Cys Arg Tyr Gln Lys Cys Leu Ala Met Gly Met Lys Arg Glu Ala
190                 195                 200

GTG CAG GAG GAG CGG CAG CGT GGC AAG GAC CGG AAC GAG AAT GAG GTG         735
Val Gln Glu Glu Arg Gln Arg Gly Lys Asp Arg Asn Glu Asn Glu Val
205                 210                 215                 220

GAG TCG ACC AGC AGC GCC AAC GAG GAC ATG CCG GTG GAG AGG ATC CTG         783
Glu Ser Thr Ser Ser Ala Asn Glu Asp Met Pro Val Glu Arg Ile Leu
                225                 230                 235

GAG GCT GAG CTG GCC GTG GAG CCC AAG ACC GAG ACC TAC GTG GAG GCA         831
Glu Ala Glu Leu Ala Val Glu Pro Lys Thr Glu Thr Tyr Val Glu Ala
            240                 245                 250

AAC ATG GGG CTG AAC CCC AGC TCG CCG AAC GAC CCT GTC ACC AAC ATT         879
Asn Met Gly Leu Asn Pro Ser Ser Pro Asn Asp Pro Val Thr Asn Il
        255                 260                 265
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TGC CAA GCA GCC GAC AAA CAG CTT TTC ACC CTG GTG GAG TGG GCC AAG 927
Cys Gln Ala Ala Asp Lys Gln Leu Phe Thr Leu Val Glu Trp Ala Lys
270 275 280

CGG ATC CCA CAC TTC TCA GAG CTG CCC CTG GAC GAC CAG GTC ATC CTG 975
Arg Ile Pro His Phe Ser Glu Leu Pro Leu Asp Asp Gln Val Ile Leu
285 290 295 300

CTG CGG GCA GGC TGG AAT GAG CTG CTC ATC GCC TCC TTC TCC CAC CGC 1023
Leu Arg Ala Gly Trp Asn Glu Leu Leu Ile Ala Ser Phe Ser His Arg
305 310 315

TCC ATC GCC GTG AAG GAC GGG ATC CTC CTG GCC ACC GGG CTG CAC GTC 1071
Ser Ile Ala Val Lys Asp Gly Ile Leu Leu Ala Thr Gly Leu His Val
320 325 330

CAC CGG AAC AGC GCC CAC AGC GCA GGG GTG GGC GCC ATC TTT GAC AGG 1119
His Arg Asn Ser Ala His Ser Ala Gly Val Gly Ala Ile Phe Asp Arg
335 340 345

GTG CTG ACG GAG CTT GTG TCC AAG ATG CGG GAC ATG CAG ATG GAC AAG 1167
Val Leu Thr Glu Leu Val Ser Lys Met Arg Asp Met Gln Met Asp Lys
350 355 360

ACG GAG CTG GGC TGC CTG CGC GCC ATC GTC CTC TTT AAC CCT GAC TCC 1215
Thr Glu Leu Gly Cys Leu Arg Ala Ile Val Leu Phe Asn Pro Asp Ser
365 370 375 380

AAG GGG CTC TCG AAC CCG GCC GAG GTG GAG GCG CTG AGG GAG AAG GTC 1263
Lys Gly Leu Ser Asn Pro Ala Glu Val Glu Ala Leu Arg Glu Lys Val
385 390 395

TAT GCG TCC TTG GAG GCC TAC TGC AAG CAC AAG TAC CCA GAG CAG CCG 1311
Tyr Ala Ser Leu Glu Ala Tyr Cys Lys His Lys Tyr Pro Glu Gln Pro
400 405 410

GGA AGG TTC GCT AAG CTC TTG CTC CGC CTG CCG GCT CTG CGC TCC ATC 1359
Gly Arg Phe Ala Lys Leu Leu Leu Arg Leu Pro Ala Leu Arg Ser Ile
415 420 425

GGG CTC AAA TGC CTG GAA CAT CTC TTC TTC TTC AAG CTC ATC GGG GAC 1407
Gly Leu Lys Cys Leu Glu His Leu Phe Phe Phe Lys Leu Ile Gly Asp
430 435 440

ACA CCC ATT GAC ACC TTC CTT ATG GAG ATG CTG GAG GCG CCG CAC CAA 1455
Thr Pro Ile Asp Thr Phe Leu Met Glu Met Leu Glu Ala Pro His Gln
445 450 455 460

ATG ACT TAGGCCTGCG GGCCCATCCT TTGTGCCCAC CCGTTCTGGC CACCCTGCCT 1511
Met Thr

GGACGCCAGC TGTTCTTCTC AGCCTGAGCC CTGTCCCTGC CCTTCTCTGC CTGGCCTGTT 1571

TGGACTTTGG GGCACAGCCT GTCACTGCTC TGCCTAAGAG ATGTGTTGTC ACCCTCCTTA 1631

TTTCTGTTAC TACTTGTCTG TGGCCCAGGG CAGTGGCTTT CCTGAGCAGC AGCCTTCGTG 1691

GCAAGAACTA GCGTGAGCCC AGCCAGGCGC CTCCCCACCG GGCTCTCAGG ACGCCCTGCC 1751

ACACCCACGG GGCTTGGGCG ACTACAGGGT CTTCGGCCCC AGCCCTGGAG CTGCAGGAGT 1811

TGGGAACGGG GCTTTTGTTT CCGTTGCTGT TTATCGATGC TGGTTTTCAG AATTC 1866

SEQ ID NO:2:

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Met Asp Thr Lys His Ph  Leu Pr  Leu Asp Phe Ser Thr Gln Val Asn
 1               5                   10                  15

Ser Ser Leu Thr Ser Pro Thr Gly Arg Gly Ser Met Ala Ala Pro Ser
            20                  25                  30

Leu His Pro Ser Leu Gly Pro Gly Ile Gly Ser Pro Gly Gln Leu His
        35                  40                  45

Ser Pro Ile Ser Thr Leu Ser Ser Pro Ile Asn Gly Met Gly Pro Pro
    50                  55                  60

Phe Ser Val Ile Ser Ser Pro Met Gly Pro His Ser Met Ser Val Pro
65                  70                  75                  80

Thr Thr Pro Thr Leu Gly Phe Ser Thr Gly Ser Pro Gln Leu Ser Ser
                85                  90                  95

Pro Met Asn Pro Val Ser Ser Ser Glu Asp Ile Lys Pro Pro Leu Gly
            100                 105                 110

Leu Asn Gly Val Leu Lys Val Pro Ala His Pro Ser Gly Asn Met Ala
        115                 120                 125

Ser Phe Thr Lys His Ile Cys Ala Ile Cys Gly Asp Arg Ser Ser Gly
    130                 135                 140

Lys His Tyr Gly Val Tyr Ser Cys Glu Gly Cys Lys Gly Phe Phe Lys
145                 150                 155                 160

Arg Thr Val Arg Lys Asp Leu Thr Tyr Thr Cys Arg Asp Asn Lys Asp
                165                 170                 175

Cys Leu Ile Asp Lys Arg Gln Arg Asn Arg Cys Gln Tyr Cys Arg Tyr
            180                 185                 190

Gln Lys Cys Leu Ala Met Gly Met Lys Arg Glu Ala Val Gln Glu Glu
        195                 200                 205

Arg Gln Arg Gly Lys Asp Arg Asn Glu Asn Glu Val Glu Ser Thr Ser
    210                 215                 220

Ser Ala Asn Glu Asp Met Pro Val Glu Arg Ile Leu Glu Ala Glu Leu
225                 230                 235                 240

Ala Val Glu Pro Lys Thr Glu Thr Tyr Val Glu Ala Asn Met Gly Leu
                245                 250                 255

Asn Pro Ser Ser Pro Asn Asp Pro Val Thr Asn Ile Cys Gln Ala Ala
            260                 265                 270

Asp Lys Gln Leu Phe Thr Leu Val Glu Trp Ala Lys Arg Ile Pro His
        275                 280                 285

Phe Ser Glu Leu Pro Leu Asp Asp Gln Val Ile Leu Leu Arg Ala Gly
    290                 295                 300

Trp Asn Glu Leu Leu Ile Ala Ser Phe Ser His Arg Ser Ile Ala Val
305                 310                 315                 320

Lys Asp Gly Ile Leu Leu Ala Thr Gly Leu His Val His Arg Asn Ser
                325                 330                 335

Ala His Ser Ala Gly Val Gly Ala Ile Phe Asp Arg Val Leu Thr Glu
            340                 345                 350

Leu Val Ser Lys Met Arg Asp Met Gln Met Asp Lys Thr Glu Leu Gly
        355                 360                 365

Cys Leu Arg Ala Ile Val Leu Phe Asn Pro Asp Ser Lys Gly Leu Ser
    370                 375                 380
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Asn Pro Ala Glu Val Glu Ala Leu Arg Glu Lys Val Tyr Ala Ser Leu
385 390 395 400

Glu Ala Tyr Cys Lys His Lys Tyr Pro Glu Gln Pro Gly Arg Phe Ala
405 410 415

Lys Leu Leu Leu Arg Leu Pro Ala Leu Arg Ser Ile Gly Leu Lys Cys
420 425 430

Leu Glu His Leu Phe Phe Phe Lys Leu Ile Gly Asp Thr Pro Ile Asp
435 440 445

Thr Phe Leu Met Glu Met Leu Glu Ala Pro His Gln Met Thr
450 455 460

SEQ ID NO:3:

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GAATTCGCGG CCGCGGCGAC TTTTGCAACA ACTCGCCGCG CCGCGGCCTC CGCGCGCCGC    60

CGCCGCCGCT GCCGCCGCCG GCTCCCCGCC GCCCGGGCCC CGGGCGGGCC GCGCCGGGGG   120

CCGCCGCGCT GCCGCCCTGC TGCTCCGCCG CCGGCTGGGC ATGAGTTAGT CGCAGAC      177

ATG GAC ACC AAA CAT TTC CTG CCG CTC GAC TTC TCT ACC CAG GTG AAC     225
Met Asp Thr Lys His Phe Leu Pro Leu Asp Phe Ser Thr Gln Val Asn
 1               5                  10                  15

TCT TCG TCC CTC AAC TCT CCA ACG GGT CGA GGC TCC ATG GCT GTC CCC     273
Ser Ser Ser Leu Asn Ser Pro Thr Gly Arg Gly Ser Met Ala Val Pro
            20                  25                  30

TCG CTG CAC CCC TCC TTG GGT CCG GGA ATC GGC TCT CCA CTG GGC TCG     321
Ser Leu His Pro Ser Leu Gly Pro Gly Ile Gly Ser Pro Leu Gly Ser
        35                  40                  45

CCT GGG CAG CTG CAC TCT CCT ATC AGC ACC CTG AGC TCC CCC ATC AAT     369
Pro Gly Gln Leu His Ser Pro Ile Ser Thr Leu Ser Ser Pro Ile Asn
    50                  55                  60

GGC ATG GGT CCG CCC TTC TCT GTC ATC AGC TCC CCC ATG GGC CCG CAC     417
Gly Met Gly Pro Pro Phe Ser Val Ile Ser Ser Pro Met Gly Pro His
 65                  70                  75                  80

TCC ATG TCG GTA CCC ACC ACA CCC ACA TTG GGC TTC GGG ACT GGT AGC     465
Ser Met Ser Val Pro Thr Thr Pro Thr Leu Gly Phe Gly Thr Gly Ser
                 85                  90                  95

CCC CAG CTC AAT TCA CCC ATG AAC CCT GTG AGC AGC ACT GAG GAT ATC     513
Pro Gln Leu Asn Ser Pro Met Asn Pro Val Ser Ser Thr Glu Asp Ile
            100                 105                 110

AAG CCG CCA CTA GGC CTC AAT GGC GTC CTC AAG GTT CCT GCC CAT CCC     561
Lys Pro Pro Leu Gly Leu Asn Gly Val Leu Lys Val Pro Ala His Pro
        115                 120                 125

TCA GGA AAT ATG GCC TCC TTC ACC AAG CAC ATC TGT GCT ATC TGT GGG     609
Ser Gly Asn Met Ala Ser Phe Thr Lys His Ile Cys Ala Ile Cys Gly
    130                 135                 140

GAC CGC TCC TCA GGC AAA CAC TAT GGG GTA TAC AGT TGT GAG GGC TGC     657
Asp Arg Ser Ser Gly Lys His Tyr Gly Val Tyr Ser Cys Glu Gly Cys
145                 150                 155                 160

AAG GGC TTC TTC AAG AGG ACA GTA CGC AAA GAC CTG ACC TAC ACC TGC     705
Lys Gly Phe Phe Lys Arg Thr Val Arg Lys Asp Leu Thr Tyr Thr Cys
                165                 170                 175

CGA GAC AAC AAG GAC TGC CTG ATG GAC AAG AGA CAG CGG AAC CGG TGT     753
Arg Asp Asn Lys Asp Cys Leu Ile Asp Lys Arg Gln Arg Asn Arg Cys
            180                 185                 190

CAG TAC TGC CGC TAC CAG AAG TGC CTG GCC ATG GGC ATG AAG CGG GAA     801
Gln Tyr Cys Arg Tyr Gln Lys Cys Leu Ala Met Gly Met Lys Arg Glu
        195                 200                 205

GCT GTG CAG GAG GAG CGG CAG CGG GGC AAG GAC CGG AAT GAG AAC GAG     849
Ala Val Gln Glu Glu Arg Gln Arg Gly Lys Asp Arg Asn Glu Asn Glu
    210                 215                 220

GTG GAG TCC ACC AGC AGT GCC AAC GAG GAC ATG CCT GTA GAG AAG ATT     897
Val Glu Ser Thr Ser Ser Ala Asn Glu Asp Met Pro Val Glu Lys Ile
225                 230                 235                 240

CTG GAA GCC GAG CTT GCT GTC GAG CCC AAG ACT GAG ACA TAC GTG GAG     945
Leu Glu Ala Glu Leu Ala Val Glu Pro Lys Thr Glu Thr Tyr Val Glu
                245                 250                 255
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GCA AAC ATG GGG CTG AAC CCC AGC TCA CCA AAT GAC CCT GTT ACC AAC      993
Ala Asn Met Gly Leu Asn Pro Ser Ser Pro Asn Asp Pro Val Thr Asn
            260                 265                 270

ATC TGT CAA GCA GCA GAC AAG CAG CTC TTC ACT CTT GTG GAG TGG GCC     1041
Il  Cys Gln Ala Ala Asp Lys Gln Leu Phe Thr Leu Val Glu Trp Ala
        275                 280                 285

AAG AGG ATC CCA CAC TTT TCT GAG CTG CCC CTA GAC GAC CAG GTC ATC     1089
Lys Arg Ile Pro His Phe Ser Glu Leu Pro Leu Asp Asp Gln Val Ile
    290                 295                 300

CTG CTA CGG GCA GGC TGG AAC GAG CTG CTG ATC GCC TCC TTC TCC CAC     1137
Leu Leu Arg Ala Gly Trp Asn Glu Leu Leu Ile Ala Ser Phe Ser His
305                 310                 315                 320

CGC TCC ATA GCT GTG AAA GAT GGG ATT CTC CTG GCC ACC GGG CTG CAC     1185
Arg Ser Ile Ala Val Lys Asp Gly Ile Leu Leu Ala Thr Gly Leu His
                325                 330                 335

GTA CAC CGG AAC AGC GCT CAC AGT GCT GGG GTG GGC GCC ATC TTT GAC     1233
Val His Arg Asn Ser Ala His Ser Ala Gly Val Gly Ala Ile Phe Asp
            340                 345                 350

AGG GTG CTA ACA GAG CTG GTG TCT AAG ATG CGT GAC ATG CAG ATG GAC     1281
Arg Val Leu Thr Glu Leu Val Ser Lys Met Arg Asp Met Gln Met Asp
        355                 360                 365

AAG ACG GAG CTG GGC TGC CTG CGA GCC ATT GTC CTG TTC AAC CCT GAC     1329
Lys Thr Glu Leu Gly Cys Leu Arg Ala Ile Val Leu Phe Asn Pro Asp
    370                 375                 380

TCT AAG GGG CTC TCA AAC CCT GCT GAG GTG GAG GCG TTG AGG GAG AAG     1377
Ser Lys Gly Leu Ser Asn Pro Ala Glu Val Glu Ala Leu Arg Glu Lys
385                 390                 395                 400

GTG TAT GCG TCA CTA GAA GCG TAC TGC AAA CAC AAG TAC CCT GAG CAG     1425
Val Tyr Ala Ser Leu Glu Ala Tyr Cys Lys His Lys Tyr Pro Glu Gln
                405                 410                 415

CCG GGC AGG TTT GCC AAG CTG CTG CTC CGC CTG CCT GCA CTG CGT TCC     1473
Pro Gly Arg Phe Ala Lys Leu Leu Leu Arg Leu Pro Ala Leu Arg Ser
            420                 425                 430

ATC GGG CTC AAG TGC CTG GAG CAC CTG TTC TTC TTC AAG CTC ATC GGG     1521
Ile Gly Leu Lys Cys Leu Glu His Leu Phe Phe Phe Lys Leu Ile Gly
        435                 440                 445

GAC ACG CCC ATC GAC ACC TTC CTC ATG GAG ATG CTG GAG GCA CCA CAT     1569
Asp Thr Pro Ile Asp Thr Phe Leu Met Glu Met Leu Glu Ala Pro His
    450                 455                 460

CAA GCC ACC TAGGCCCCCG CCGCCGTGTG CCGGTCCCGT GCCCTGCCTG             1618
Gln Ala Thr
465

GACACAGCTG CTCAGCTCCA GCCCTGCCCC TGCCCTTTCT GATGGCCCGT GTGGATCTTT    1678

GGGGTGCAGT GTCCTTATGG GCCCAAAAGA TGCATCACCA TCCTCGCCAT CTTTACTCAT    1738

GCTTGCCTTT GGCCCAGGGC ATAGCAGAGC TGGTGTGACA CCTGGCCAGC TCCTGCCCTA    1798

CATCAGGCTC TAAGGCTATG CTGCTGTCAC CCCGAGGGTC GTGGGGTTCG TCATGGGGCC    1858

TTCAGCACCT GGAGCTGCAA GAGCTGGGAA AAGGGCTTGT TCTGGTTGCT GGTTGCTGGT    1918

CGCTGGTTCT CGACATCCCA CATGGCACCT CTGTTTGGAG TGCCCCATCT TGGCCTGTTC    1978

AGAGTCCTGG TACCCAGTTA GGGTGGGAAT CCACCTGGGA TCAAGAAGGA GCAGGTGGGG    2038

CAGGCCGTAT CCTCCTGGGT CATAGCTAAC CTATAAAGGC GCCGCGAATT CCTCGAG       2095
```

SEQ ID NO:4:

Met Asp Thr Lys His Phe Leu Pro Leu Asp Phe Ser Thr Gln Val Asn
1 5 10 15

Ser Ser Ser Leu Asn Ser Pro Thr Gly Arg Gly Ser Met Ala Val Pro
20 25 30

Ser Leu His Pro Ser Leu Gly Pro Gly Ile Gly Ser Pro Leu Gly Ser
35 40 45

Pro Gly Gln Leu His Ser Pro Ile Ser Thr Leu Ser Ser Pro Ile Asn
50 55 60

Gly Met Gly Pro Pro Phe Ser Val Ile Ser Ser Pro Met Gly Pro His
65 70 75 80

Ser Met Ser Val Pro Thr Thr Pro Thr Leu Gly Phe Gly Thr Gly Ser
85 90 95

Pro Gln Leu Asn Ser Pro Met Asn Pro Val Ser Ser Thr Glu Asp Ile
100 105 110

Lys Pro Pro Leu Gly Leu Asn Gly Val Leu Lys Val Pro Ala His Pro
115 120 125

Ser Gly Asn Met Ala Ser Phe Thr Lys His Ile Cys Ala Ile Cys Gly
130 135 140

Asp Arg Ser Ser Gly Lys His Tyr Gly Val Tyr Ser Cys Glu Gly Cys
145 150 155 160

Lys Gly Phe Phe Lys Arg Thr Val Arg Lys Asp Leu Thr Tyr Thr Cys
165 170 175

Arg Asp Asn Lys Asp Cys Leu Ile Asp Lys Arg Gln Arg Asn Arg Cys
180 185 190

Gln Tyr Cys Arg Tyr Gln Lys Cys Leu Ala Met Gly Met Lys Arg Glu
195 200 205

Ala Val Gln Glu Glu Arg Gln Arg Gly Lys Asp Arg Asn Glu Asn Glu
210 215 220

Val Glu Ser Thr Ser Ser Ala Asn Glu Asp Met Pro Val Glu Lys Ile
225 230 235 240

Leu Glu Ala Glu Leu Ala Val Glu Pro Lys Thr Glu Thr Tyr Val Glu
245 250 255

Ala Asn Met Gly Leu Asn Pro Ser Ser Pro Asn Asp Pro Val Thr Asn
260 265 270

Ile Cys Gln Ala Ala Asp Lys Gln Leu Phe Thr Leu Val Glu Trp Ala
275 280 285

Lys Arg Ile Pro His Phe Ser Glu Leu Pro Leu Asp Asp Gln Val Ile
290 295 300

Leu Leu Arg Ala Gly Trp Asn Glu Leu Leu Ile Ala Ser Phe Ser His
305 310 315 320

Arg Ser Ile Ala Val Lys Asp Gly Ile Leu Leu Ala Thr Gly Leu His
325 330 335

Val His Arg Asn Ser Ala His Ser Ala Gly Val Gly Ala Ile Phe Asp
340 345 350

Arg Val Leu Thr Glu Leu Val Ser Lys Met Arg Asp Met Gln Met Asp
355 360 365

Lys Thr Glu Leu Gly Cys Leu Arg Ala Il Val Leu Phe Asn Pro Asp
370 375 380

```
Ser Lys Gly Leu Ser Asn Pro Ala Glu Val Glu Ala Leu Arg Glu Lys
385                 390                 395                 400

Val Tyr Ala Ser Leu Glu Ala Tyr Cys Lys His Lys Tyr Pro Glu Gln
                405                 410                 415

Pro Gly Arg Phe Ala Lys Leu Leu Leu Arg Leu Pro Ala Leu Arg Ser
            420                 425                 430

Ile Gly Leu Lys Cys Leu Glu His Leu Phe Phe Phe Lys Leu Ile Gly
        435                 440                 445

Asp Thr Pro Ile Asp Thr Phe Leu Met Glu Met Leu Glu Ala Pro His
    450                 455                 460

Gln Ala Thr
465
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SEQ ID NO:5:

GAATTCGCGG CCGCGCTGTG CCTGGGAGCC GAGAGAGAGA GAGAGAGAGA GAGAGAGAGA 60

GAGAGAGAGA GAGAGGCTGT ACTCTTCAGA AGCGCACGAG AGGAATGAAC TGAGCAGCCA 120

AC ATG TAT GGA AAT TAT TCC CAC TTC ATG AAG TTT CCC ACC GGC TTT 167
Met Tyr Gly Asn Tyr Ser His Phe Met Lys Phe Pro Thr Gly Phe
1 5 10 15

GGT GGC TCC CCT GGT CAC ACT GGC TCG ACG TCC ATG AGC CCT TCA GTA 215
Gly Gly Ser Pro Gly His Thr Gly Ser Thr Ser Met Ser Pro Ser Val
20 25 30

GCC TTG CCC ACG GGG AAG CCA ATG GAC AGC CAC CCC AGC TAC ACA GAC 263
Ala Leu Pro Thr Gly Lys Pro Met Asp Ser His Pro Ser Tyr Thr Asp
35 40 45

ACC CCA GTG AGT GCC CCT CGG ACG CTG AGT GCT GTG GGA ACC CCC CTC 311
Thr Pro Val Ser Ala Pro Arg Thr Leu Ser Ala Val Gly Thr Pro Leu
50 55 60

AAT GCT CTT GGC TCT CCG TAT AGA GTC ATC ACT TCT GCC ATG GGT CCA 359
Asn Ala Leu Gly Ser Pro Tyr Arg Val Ile Thr Ser Ala Met Gly Pro
65 70 75

CCC TCA GGA GCA CTG GCA GCT CCT CCA GGA ATC AAC TTG GTG GCT CCA 407
Pro Ser Gly Ala Leu Ala Ala Pro Pro Gly Ile Asn Leu Val Ala Pro
80 85 90 95

CCC AGC TCC CAG CTA AAT GTG GTC AAC AGT GTC AGC AGC TCT GAG GAC 455
Pro Ser Ser Gln Leu Asn Val Val Asn Ser Val Ser Ser Ser Glu Asp
100 105 110

ATC AAG CCC TTA CCA GGT CTG CCT GGG ATT GGA AAT ATG AAC TAC CCA 503
Ile Lys Pro Leu Pro Gly Leu Pro Gly Ile Gly Asn Met Asn Tyr Pro
115 120 125

TCC ACC AGC CCT GGG TCT CTG GTG AAA CAC ATC TGT GCC ATC TGT GGG 551
Ser Thr Ser Pro Gly Ser Leu Val Lys His Ile Cys Ala Ile Cys Gly
130 135 140

GAC AGA TCC TCA GGG AAG CAC TAC GGT GTG TAC AGC TGT GAA GGT TGC 599
Asp Arg Ser Ser Gly Lys His Tyr Gly Val Tyr Ser Cys Glu Gly Cys
145 150 155

AAA GGC TTC TTC AAA AGG ACC ATC AGG AAA GAT CTC ATC TAC ACC TGT 647
Lys Gly Phe Phe Lys Arg Thr Ile Arg Lys Asp Leu Ile Tyr Thr Cys
160 165 170 175

CGG GAT AAC AAA GAT TGT CTC ATC GAC AAG CGC CAG CGC AAC CGC TGC 695
Arg Asp Asn Lys Asp Cys Leu Ile Asp Lys Arg Gln Arg Asn Arg Cys
180 185 190

CAG TAC TGT CGC TAC CAG AAG TGC CTG GTC ATG GGC ATG AAG CGG GAA 743
Gln Tyr Cys Arg Tyr Gln Lys Cys Leu Val Met Gly Met Lys Arg Glu
195 200 205

GCT GTG CAA GAA GAA AGG CAG AGG AGC CGA GAG CGA GCA GAG AGT GAG 791
Ala Val Gln Glu Glu Arg Gln Arg Ser Arg Glu Arg Ala Glu Ser Glu
210 215 220

GCA GAA TGT GCC AGT AGT AGC CAC GAA GAC ATG CCC GTG GAG AGG ATT 839
Ala Glu Cys Ala Ser Ser Ser His Glu Asp Met Pro Val Glu Arg Ile
225 230 235

CTA GAA GCC GAA CTT GCT GTG GAA CCA AAG ACA GAA TCC TAC GGT GAC 887
Leu Glu Ala Glu Leu Ala Val Glu Pro Lys Thr Glu Ser Tyr Gly Asp
240 245 250 255

ATG AAC GTG GAG AAC TCA ACA AAT GAC CCT GTT ACC AAC ATA TGC CAT 935
Met Asn Val Glu Asn Ser Thr Asn Asp Pro Val Thr Asn Ile Cys His
260 265 270

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GCT GCA GAT AAG CAA CTT TTC ACC CTC GTT GAG TGG GCC AAA CGC ATC      983
Ala Ala Asp Lys Gln Leu Phe Thr Leu Val Glu Trp Ala Lys Arg Il
            275                     280                     285

CCC CAC TTC TCA GAT CTC ACC TTG GAG GAC CAG GTC ATT CTA CTC CGG     1031
Pro His Phe Ser Asp Leu Thr Leu Glu Asp Gln Val Ile Leu Leu Arg
        290                     295                     300

GCA GGG TGG AAT GAA CTG CTC ATT GCC TCC TTC TCC CAC CGC TCG GTT     1079
Ala Gly Trp Asn Glu Leu Leu Ile Ala Ser Phe Ser His Arg Ser Val
    305                     310                     315

TCC GTC CAG GAT GGC ATC CTG CTG GCC ACG GGC CTC CAC GTG CAC AGG     1127
Ser Val Gln Asp Gly Ile Leu Leu Ala Thr Gly Leu His Val His Arg
320                     325                     330                     335

AGC AGC GCT CAC AGC CGG GGA GTC GGC TCC ATC TTC GAC AGA GTC CTT     1175
Ser Ser Ala His Ser Arg Gly Val Gly Ser Ile Phe Asp Arg Val Leu
                340                     345                     350

ACA GAG TTG GTG TCC AAG ATG AAA GAC ATG CAG ATG GAT AAG TCA GAG     1223
Thr Glu Leu Val Ser Lys Met Lys Asp Met Gln Met Asp Lys Ser Glu
            355                     360                     365

CTG GGG TGC CTA CGG GCC ATC GTG CTG TTT AAC CCA GAT GCC AAG GGT     1271
Leu Gly Cys Leu Arg Ala Ile Val Leu Phe Asn Pro Asp Ala Lys Gly
        370                     375                     380

TTA TCC AAC CCC TCT GAG GTG GAG ACT CTT CGA GAG AAG GTT TAT GCC     1319
Leu Ser Asn Pro Ser Glu Val Glu Thr Leu Arg Glu Lys Val Tyr Ala
    385                     390                     395

ACC CTG GAG GCC TAT ACC AAG CAG AAG TAT CCG GAA CAG CCA GGC AGG     1367
Thr Leu Glu Ala Tyr Thr Lys Gln Lys Tyr Pro Glu Gln Pro Gly Arg
400                     405                     410                     415

TTT GCC AAG CTT CTG CTG CGT CTC CCT GCT CTG CGC TCC ATC GGC TTG     1415
Phe Ala Lys Leu Leu Leu Arg Leu Pro Ala Leu Arg Ser Ile Gly Leu
                420                     425                     430

AAA TGC CTG GAA CAC CTC TTC TTC TTC AAG CTC ATT GGA GAC ACT CCC     1463
Lys Cys Leu Glu His Leu Phe Phe Phe Lys Leu Ile Gly Asp Thr Pro
            435                     440                     445

ATC GAC AGC TTC CTC ATG GAG ATG TTG GAG ACC CCA CTG CAG ATC ACC     1511
Ile Asp Ser Phe Leu Met Glu Met Leu Glu Thr Pro Leu Gln Ile Thr
        450                     455                     460

TGAACCTCCT CAGCTGCAGC TTCCCCACCC AGGGTGACCC TTGGGCGGGT GTGTGTGTGT     1571

GGCCCTACCC TGCACACTCT CCCCCATCTT CCACTCTGGC CTCCCTTCCT GTCCCCAAAA     1631

TGTGATGCTT GTAATAAGCG GCCGCGAATT C                                    1662
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SEQ ID NO:6:

Met Tyr Gly Asn Tyr Ser His Phe Met Lys Phe Pro Thr Gly Phe Gly
1 5 10 15

Gly Ser Pro Gly His Thr Gly Ser Thr Ser Met Ser Pro Ser Val Ala
20 25 30

Leu Pro Thr Gly Lys Pro Met Asp Ser His Pro Ser Tyr Thr Asp Thr
35 40 45

Pro Val Ser Ala Pro Arg Thr Leu Ser Ala Val Gly Thr Pro Leu Asn
50 55 60

Ala Leu Gly Ser Pro Tyr Arg Val Ile Thr Ser Ala Met Gly Pro Pro
65 70 75 80

Ser Gly Ala Leu Ala Ala Pro Pro Gly Ile Asn Leu Val Ala Pro Pro
85 90 95

Ser Ser Gln Leu Asn Val Val Asn Ser Val Ser Ser Ser Glu Asp Ile
100 105 110

Lys Pro Leu Pro Gly Leu Pro Gly Ile Gly Asn Met Asn Tyr Pro Ser
115 120 125

Thr Ser Pro Gly Ser Leu Val Lys His Ile Cys Ala Ile Cys Gly Asp
130 135 140

Arg Ser Ser Gly Lys His Tyr Gly Val Tyr Ser Cys Glu Gly Cys Lys
145 150 155 160

Gly Phe Phe Lys Arg Thr Ile Arg Lys Asp Leu Ile Tyr Thr Cys Arg
165 170 175

Asp Asn Lys Asp Cys Leu Ile Asp Lys Arg Gln Arg Asn Arg Cys Gln
180 185 190

Tyr Cys Arg Tyr Gln Lys Cys Leu Val Met Gly Met Lys Arg Glu Ala
195 200 205

Val Gln Glu Glu Arg Gln Arg Ser Arg Glu Arg Ala Glu Ser Glu Ala
210 215 220

Glu Cys Ala Ser Ser Ser His Glu Asp Met Pro Val Glu Arg Ile Leu
225 230 235 240

Glu Ala Glu Leu Ala Val Glu Pro Lys Thr Glu Ser Tyr Gly Asp Met
245 250 255

Asn Val Glu Asn Ser Thr Asn Asp Pro Val Thr Asn Ile Cys His Ala
260 265 270

Ala Asp Lys Gln Leu Phe Thr Leu Val Glu Trp Ala Lys Arg Ile Pro
275 280 285

His Phe Ser Asp Leu Thr Leu Glu Asp Gln Val Ile Leu Leu Arg Ala
290 295 300

Gly Trp Asn Glu Leu Leu Ile Ala Ser Phe Ser His Arg Ser Val Ser
305 310 315 320

Val Gln Asp Gly Ile Leu Leu Ala Thr Gly Leu His Val His Arg Ser
325 330 335

Ser Ala His Ser Arg Gly Val Gly Ser Ile Phe Asp Arg Val Leu Thr
340 345 350

Glu Leu Val Ser Lys Met Lys Asp Met Gln Met Asp Lys Ser Glu Leu
355 360 365

Gly Cys Leu Arg Ala Ile Val Leu Phe Asn Pro Asp Ala Lys Gly Leu
370 375 380

Ser Asn Pro Ser Glu Val Glu Thr Leu Arg Glu Lys Val Tyr Ala Thr
385 390 395 400

Leu Glu Ala Tyr Thr Lys Gln Lys Tyr Pro Glu Gln Pro Gly Arg Phe
405 410 415

Ala Lys Leu Leu Leu Arg Leu Pro Ala Leu Arg Ser Ile Gly Leu Lys
420 425 430

Cys Leu Glu His Leu Phe Phe Phe Lys Leu Ile Gly Asp Thr Pro Ile
435 440 445

Asp Ser Phe Leu Met Glu Met Leu Glu Thr Pro Leu Gln Ile Thr
450 455 460

SEQ ID NO:7:

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GAATTCCCCC GAAGCCCAGA CAGCTCCTCC CCAAATCCCC TTTCTCAGGG GATCCGTCCG      60

TCTTCTCCTC CTGGCCCACC TCTTACCCCT TCAGCACCTC CACCTCCA ATG CCA CCC      117
                                                     Met Pro Pro
                                                      1

CCG CCA CTG GGC TCC CCC TTC CCA GTC ATC AGT TCT TCC ATG GGG TCC      165
Pro Pro Leu Gly Ser Pro Phe Pro Val Ile Ser Ser Ser Met Gly Ser
     5                  10                  15

CCT GGT CTG CCC CCT CCG GCT CCC CCA GGA TTC TCC GGG CCT GTC AGC      213
Pro Gly Leu Pro Pro Pro Ala Pro Pro Gly Phe Ser Gly Pro Val Ser
 20                  25                  30                  35

AGC CCT CAG ATC AAC TCC ACA GTG TCG CTC CCT GGG GGT GGG TCT GGC      261
Ser Pro Gln Ile Asn Ser Thr Val Ser Leu Pro Gly Gly Gly Ser Gly
                 40                  45                  50

CCC CCT GAA GAT GTG AAG CCA CCG GTC TTA GGG GTC CGG GGC CTG CAC      309
Pro Pro Glu Asp Val Lys Pro Pro Val Leu Gly Val Arg Gly Leu His
             55                  60                  65

TGT CCA CCC CCT CCA GGT GGT CCT GGG GCT GGC AAA CGG CTC TGT GCA      357
Cys Pro Pro Pro Pro Gly Gly Pro Gly Ala Gly Lys Arg Leu Cys Ala
         70                  75                  80

ATC TGC GGG GAC CGA AGC TCA GGC AAG CAC TAT GGG GTT TAC AGC TGC      405
Ile Cys Gly Asp Arg Ser Ser Gly Lys His Tyr Gly Val Tyr Ser Cys
     85                  90                  95

GAG GGC TGC AAG GGT TTC TTC AAG CGC ACC ATT CGG AAG GAC CTG ACC      453
Glu Gly Cys Lys Gly Phe Phe Lys Arg Thr Ile Arg Lys Asp Leu Thr
100                 105                 110                 115

TAC TCG TGT CGT GAT AAC AAA GAC TGT ACA GTG GAC AAG CGC CAG CGG      501
Tyr Ser Cys Arg Asp Asn Lys Asp Cys Thr Val Asp Lys Arg Gln Arg
                120                 125                 130

AAT CGC TGT CAG TAC TGT CGC TAT CAG AAG TGC CTG GCC ACT GGC ATG      549
Asn Arg Cys Gln Tyr Cys Arg Tyr Gln Lys Cys Leu Ala Thr Gly Met
            135                 140                 145

AAA AGG GAG GCG GTT CAG GAG GAG CGT CAA CGG GGG AAG GAC AAA GAC      597
Lys Arg Glu Ala Val Gln Glu Glu Arg Gln Arg Gly Lys Asp Lys Asp
        150                 155                 160

GGG GAT GGA GAT GGG GCT GGG GGA GCC CCT GAG GAG ATG CCT GTG GAC      645
Gly Asp Gly Asp Gly Ala Gly Gly Ala Pro Glu Glu Met Pro Val Asp
    165                 170                 175

AGG ATC CTG GAG GCA GAG CTT GCT GTG GAG CAG AAG AGT GAC CAA GGC      693
Arg Ile Leu Glu Ala Glu Leu Ala Val Glu Gln Lys Ser Asp Gln Gly
180                 185                 190                 195

GTT GAG GGT CCT GGG GCC ACC GGG GGT GGT GGC AGC AGC CCA AAT GAC      741
Val Glu Gly Pro Gly Ala Thr Gly Gly Gly Gly Ser Ser Pro Asn Asp
                200                 205                 210

CCA GTG ACT AAC ATC TGC CAG GCA GCT GAC AAA CAG CTG TTC ACA CTC      789
Pro Val Thr Asn Ile Cys Gln Ala Ala Asp Lys Gln Leu Phe Thr Leu
            215                 220                 225

GTT GAG TGG GCA AAG AGG ATC CCG CAC TTC TCC TCC CTA CCT CTG GAC      837
Val Glu Trp Ala Lys Arg Ile Pro His Phe Ser Ser Leu Pro Leu Asp
        230                 235                 240

GAT CAG GTC ATA CTG CTG CGG GCA GGC TGG AAC GAG CTC CTC ATT GCG      885
Asp Gln Val Ile Leu Leu Arg Ala Gly Trp Asn Glu Leu Leu Ile Ala
    245                 250                 255
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TCC TTC TCC CAT CGG TCC ATT GAT GTC CGA GAT GGC ATC CTC CTG GCC 933
Ser Phe Ser His Arg Ser Ile Asp Val Arg Asp Gly Ile Leu Leu Ala
260 265 270 275

ACG GGT CTT CAT GTG CAC AGA AAC TCA GCC CAT TCC GCA GGC GTG GGA 981
Thr Gly Leu His Val His Arg Asn Ser Ala His Ser Ala Gly Val Gly
280 285 290

GCC ATC TTT GAT CGG GTG CTG ACA GAG CTA GTG TCC AAA ATG CGT GAC 1029
Ala Ile Phe Asp Arg Val Leu Thr Glu Leu Val Ser Lys Met Arg Asp
295 300 305

ATG AGG ATG GAC AAG ACA GAG CTT GGC TGC CTG CGG GCA ATC ATA CTG 1077
Met Arg Met Asp Lys Thr Glu Leu Gly Cys Leu Arg Ala Ile Ile Leu
310 315 320

TTT AAT CCA GAC GCC AAG GGC CTC TCC AAC CCT GGA GAG GTG GAG ATC 1125
Phe Asn Pro Asp Ala Lys Gly Leu Ser Asn Pro Gly Glu Val Glu Ile
325 330 335

CTT CGG GAG AAG GTG TAC GCC TCA CTG GAG ACC TAT TGC AAG CAG AAG 1173
Leu Arg Glu Lys Val Tyr Ala Ser Leu Glu Thr Tyr Cys Lys Gln Lys
340 345 350 355

TAC CCT GAG CAG CAG GGC CGG TTT GCC AAG CTG CTG TTA CGT CTT CCT 1221
Tyr Pro Glu Gln Gln Gly Arg Phe Ala Lys Leu Leu Leu Arg Leu Pro
360 365 370

GCC CTC CGC TCC ATC GGC CTC AAG TGT CTG GAG CAC CTG TTC TTC TTC 1269
Ala Leu Arg Ser Ile Gly Leu Lys Cys Leu Glu His Leu Phe Phe Phe
375 380 385

AAG CTC ATT GGC GAC ACC CCC ATT GAC ACC TTC CTC ATG GAG ATG CTT 1317
Lys Leu Ile Gly Asp Thr Pro Ile Asp Thr Phe Leu Met Glu Met Leu
390 395 400

GAG GCT CCC CAC CAG CTA GCC TGAGCCCAGA TGCACACCGA GTGTCACTGA 1368
Glu Ala Pro His Gln Leu Ala
405 410

GGAGGACTTG AGCCTGGGCA GGGGGCAGAG CCATGGGACA GGTGCAGAGC AGGAGGGGAC 1428

TTGCCCAGCC TGCCAGGGAT CTGGCAACAC TTAGCAGGGT TCGCTTGGTC TCCAAGTCGA 1488

AGGGGACCCC AGATCCCTGT GAGGACTTTA TGTCTACCTT CAGTGGCCTT GAGTCTCTGA 1548

ATTTGTCGGG GTCTCCCATG GTGCAGGTGA TTCTTCATCC TGGCTCCCCA GCACAAAGCA 1608

CTGCCCTGCT TCCTTCTCAT TTGGCCTCAC TCCCTTCTGA AGAGTGGAAC AGAGCTCCCC 1668

CAGAAAGGGG TGTTGTGGGG CAGGCCCCCC AAGCTGATGA TCATGGGAGC AGGGCTCTGA 1728

CAGCCTTTAT CCTCTCAGAC TTGACAGATG GGGGCAGAGG AGGGACCTGC CTCTGTCTCC 1788

TGTCAGCCCC ATTTCCACAG TCCCTCCTGC AGTCAGACTG AAGAATAAAG GGGTAGTGAA 1848

GGGGCTGCTG GAGGTGGAGG AACCCATTGC TCTTTTAATT TCCTGTGAGG AGAGACTGGG 1908

AGTTAGACTC AAAGAAGTAC TGTACATCCC CAGGTTGACT TAAATGTCAG GGCTGGAGAT 1968

GGCATGTGGG CAAGGAGGCC CCTCAGGTGG GCTGTCCCAA AGCTCCCTGG GCTCTGCCTC 2028

GGGTGGCCCT ACAGCTCTTC CCTAGTCTTA AGCACAGCTA GGCTGGGAGC AAGTGGGGAC 2088

ATTGATGGGG GTGGCCAGCC TGCAGAGTTG GGTGCTGGGC TGCATGGTTT TTGCCCTGGA 2148

CCTCTTTTGG GGGTTCCCTC CCATCTTTCA CTTGCACATA AAGTTGCTTT CCAGTTAAAA 2208

AAAAAAAAA A 2219

CLAIMS

That which is claim d is:

1. A substantially pure DNA sequence which encodes a polypeptide, wherein said polypeptide is characterized by:

(1) being responsive to the presence of retinoid(s) to regulate the transcription of associated gene(s);

(2) having a DNA binding domain of about 66 amino acids with 9 Cys residues, wherein said DNA binding domain has:

(a) less than about 65 % amino acid identity with the DNA binding domain of hRAR-alpha,

(b) less than about 55 % amino acid identity with the DNA binding domain of hTR-beta, and

(c) less than about 55 % amino acid identity with the DNA binding domain of hGR; and

(3) not including the sequence set forth in Sequence ID No 7.

2. A DNA sequence according to Claim 1 wherein said polypeptide is encoded by a continuous sequence which encodes substantially the same sequence as that of:

amino acids 1 - 462 shown in Sequence ID No. 2 [hRXR-α],

amino acids 1 - 467 shown in Sequence ID No. 4 [mRXR-α], or

amino acids 1 - 463 shown in Sequence ID No. 6 [mRXR-γ].

3. A DNA sequenc according to Claim 1 wherein said polypeptide is enc d d by a continuous sequence which encodes substantially th same sequence as that of:

amino acids 135 - 200 sh wn in Sequenc ID No. 2 [hRXR-α],

amino acids 140 - 205 shown in Sequenc ID No. 4 [mRXR-α], or

amino acids 139 - 204 shown in Sequence ID No. 6 [mRXR-γ].

4. A DNA sequence according to Claim 1 which comprises a segment having a continuous nucleotide sequence which is substantially the same as:

nucleotides 76 - 1464 shown in Sequence ID No. 1 [hRXR-α],

nucleotides 181 - 1581 shown in Sequence ID No. 2 [mRXR-α], or

nucleotides 123 - 1514 shown in Sequence ID No. 3 [mRXR-γ].

5. A DNA sequence according to Claim 4 which is pSK(hRXR-alpha), pSK(mRXR-alpha), or pSK(mRXR-gamma).

6. A substantially pure DNA construct comprising:

(i) the DNA sequence of Claim 1 operatively linked to

(ii) regulatory element(s) operative for transcription of said DNA sequence and expression of said polypeptide in an animal cell in culture.

7. A DNA construct according to Claim 6 which is selected from A5C-hRXR-alpha, A5C-mRXR-alpha, A5C-mRXR-gamma, RS-hRXR-alpha, RS-mRXR-alpha, or RS-mRXR-gamma.

8. An animal cell in culture which is transformed with a DNA construct acc rding to Claim 6.

9. A cell according to Claim 8 wherein said cell is an insect cell or a mammalian cell.

10. A cell according to Claim 9 wherein the DNA construct is selected from A5C-hRXR-alpha, A5C-mRXR-alpha, A5C-mRXR-gamma, RS-hRXR-alpha, RS-mRXR-alpha, or RS-mRXR-gamma.

11. A cell according to Claim 8, wherein said cell is further transformed with a reporter vector which comprises:

(a) a promoter that is operable in said cell,

(b) a hormone response element, and

(c) a DNA segment encoding a reporter protein,

wherein said reporter protein-encoding DNA segment is operatively linked to said promoter for transcription of said DNA segment, and

wherein said hormone response element is operatively linked to said promoter for activation thereof.

12. A cell according to Claim 11 wherein:

the promoter is the 5'-LTR promoter of a mouse mammary tumor virus,

the hormone response element is selected from TRE_p or beta-RARE, and

the reporter protein is selected from chloramphenicol acetyltransferase, luciferase, or beta-galactosidase.

13. A cell according to Claim 12 wherein the reporter vector is selected from delta-MTV-TRE_p-CAT, delta-TK-TRE_p-CAT, delta-SV-TRE_p-CAT, delta-MTV-TRE_p-LUC, delta-TK-TRE_p-LUC, or d lta-SV-TRE_p-LUC.

14. A cell according to Claim 12 wher in the rep rter vector is selected from ADH-TRE$_p$-CAT, ADH-TRE$_p$-LUC, TK-TRE$_p$-CAT, or TK-TRE$_p$-LUC.

15. A cell according to Claim 14 which is a Drosophila melanogaster Schneider line 2 cell.

16. A method of testing a compound for its ability to regulate transcription-activating effects of a receptor polypeptide, said method comprising assaying for the presence or absence of reporter protein upon contacting of cells containing a receptor polypeptide and reporter vector with said compound;

wherein said receptor polypeptide is characterized by:

(1) being responsive to the presence of retinoid(s) to regulate the transcription of associated gene(s); and

(2) having a DNA binding domain of about 66 amino acids with 9 Cys residues, wherein said DNA binding domain has:

(a) less than about 65 % amino acid identity with the DNA binding domain of hRAR-alpha,

(b) less than about 55 % amino acid identity with the DNA binding domain of hTR-beta, and

(c) less than about 55 % amino acid identity with the DNA binding domain of hGR, and

wherein said reporter vector comprises:

(a) a promoter that is operable in said cell,

(b) a hormone response element, and

(c) a DNA s gment ncoding a report r protein,

wh r in said reporter protein-encoding DNA segm nt is operatively linked to said promoter for transcription of said DNA segment, and

wherein said hormone response element is operatively linked to said promoter for activation thereof.

17. A method according to Claim 16 wherein said contacting is carried out in the further presence of at least one retinoid species.

18. A method according to Claim 16 wherein the cells employed are CV-1 cells co-transformed with a vector capable of expressing said receptor polypeptide, wherein said vector is selected from RS-hRXR-alpha, RS-mRXR-alpha, or RS-mRXR-gamma and a reporter vector selected from delta-MTV-TRE$_p$-CAT, delta-TK-TRE$_p$-CAT, delta-SV-TRE$_p$-CAT, delta-MTV-TRE$_p$-LUC, delta-TK-TRE$_p$-LUC, or delta-SV-TRE$_p$-LUC.

19. A method according to Claim 16 wherein the cells employed are Drosophila melanogaster Schneider line 2 cells co-transformed with a vector capable of expressing said receptor polypeptide, wherein said vector is selected from A5C-hRXR-alpha, A5C-mRXR-alpha, or A5C-mRXR-gamma, and a reporter vector selected from ADH-TRE$_p$-CAT, ADH-TRE$_p$-LUC, TK-TRE$_p$-CAT, or TK-TRE$_p$-LUC.

20. A labeled single-stranded nucleic acid sequence, comprising at least 20 contiguous bases in length having substantially the same sequence as any 20 or more contiguous bases selected from:

(i) bases 2 - 1861, inclusive, of the DNA illustrated in Sequ nc ID No. 1 [hRXR-α], or

(ii) bas s 20 - 2095, inclusiv , of the DNA illustrated in Sequence ID No. 2 [mRXR-α], or

(iii) bases 15 - 1653, inclusive, of the DNA illustrated in Sequence ID No. 3 [mRXR-γ], or

(iv) the compl ment of any one of the sequences according to (i), (ii), or (iii).

21. A nucleic acid according to Claim 20 which is labelled with ^{32}P.

22. A method of making a receptor polypeptide, wherein said polypeptide is characterized by:

(1) being responsive to the presence of retinoid(s) to regulate the transcription of associated gene(s); and

(2) having a DNA binding domain of about 66 amino acids with 9 Cys residues, wherein said DNA binding domain has:

(a) less than about 65 % amino acid identity with the DNA binding domain of hRAR-alpha,

(b) less than about 55 % amino acid identity with the DNA binding domain of hTR-beta, and

(c) less than about 55 % amino acid identity with the DNA binding domain of hGR;

said method comprising culturing cells containing an expression vector operable in said cells to express a DNA sequence encoding said polypeptide.

23. A method according to Claim 22 wherein said receptor polypeptide has substantially the same sequence as that of:

amino acids 1 - 462 shown in Sequence ID No. 2 [hRXR-α],

amino acids 1 - 467 shown in Sequence ID N . 4 [mRXR-α], r

amino acids 1 - 463 shown in S quence ID No. 6 [mRXR-γ].

24. A method according to Claim 22 wherein said receptor polypeptide comprises a DNA binding domain with substantially the same sequence as that of:

amino acids 135 - 200 shown in Sequence ID No. 2 [hRXR-α],

amino acids 140 - 205 shown in Sequence ID No. 4 [mRXR-α], or

amino acids 139 - 204 shown in Sequence ID No. 6 [mRXR-γ].

25. A method according to Claim 22 wherein said DNA sequence comprises a segment with substantially the same nucleotide sequence as that of:

nucleotides 76 - 1464 shown in Sequence ID No. 1 [hRXR-α],

nucleotides 181 - 1581 shown in Sequence ID No. 2 [mRXR-α], or

nucleotides 123 - 1514 shown in Sequence ID No. 3 [mRXR-γ].

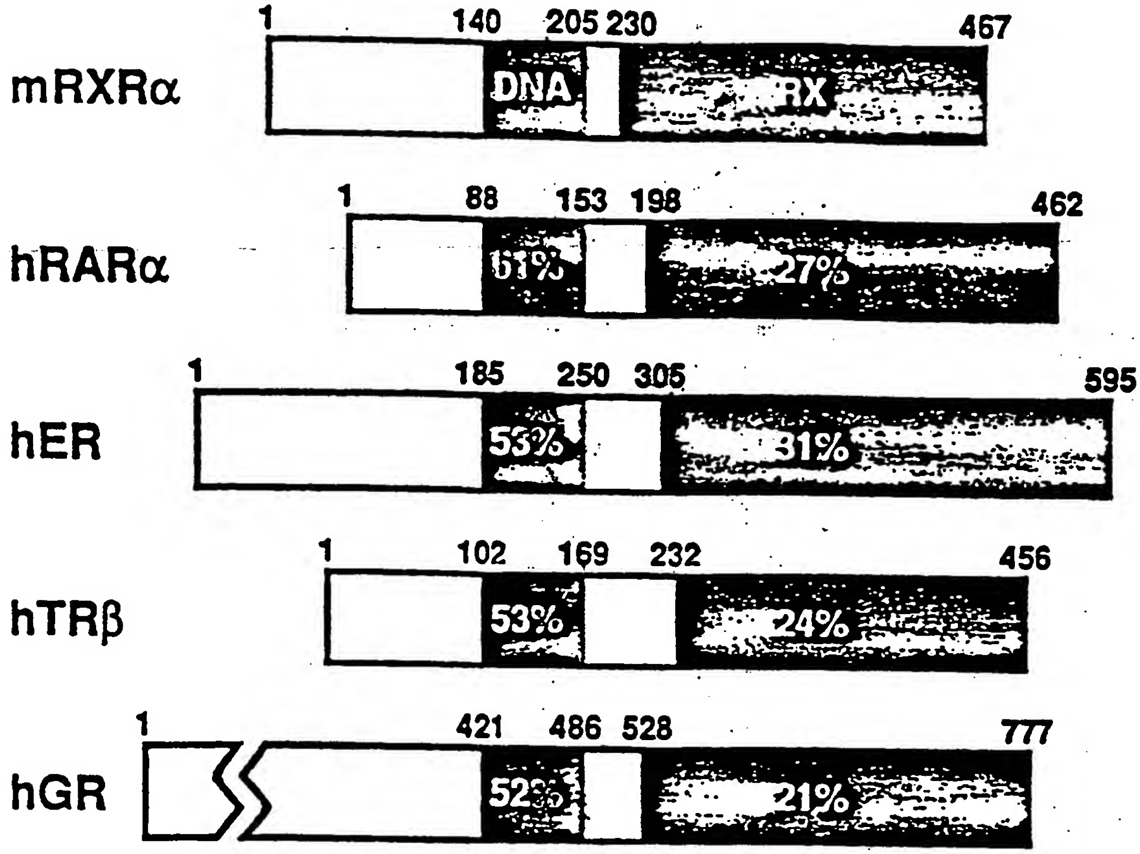
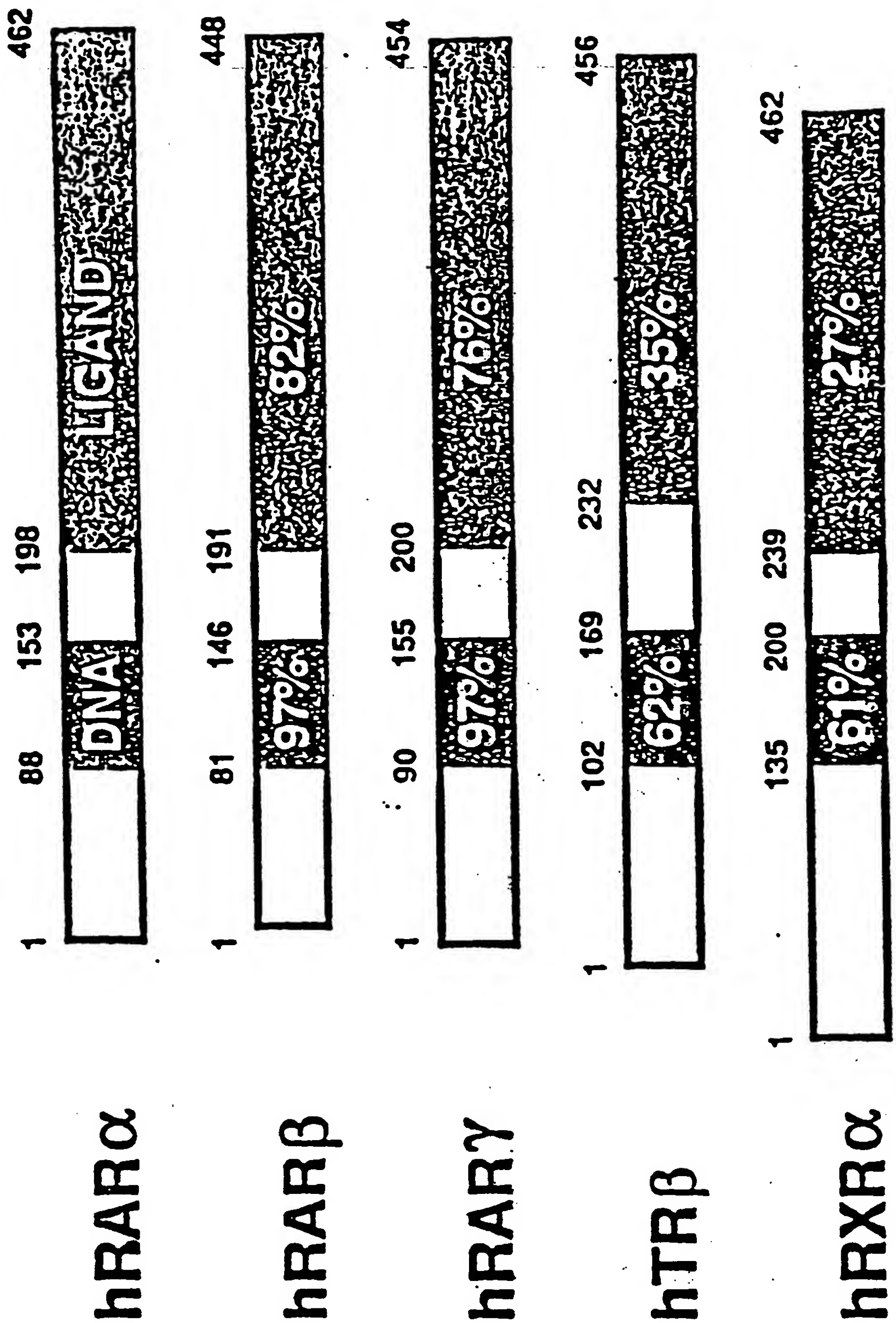

mRXRα
1
140
205
230
467
DNA
RX
hRARα
1
88
153
198
462
27%
hER
1
185
250
305
595
53%
31%
hTRβ
1
102
169
232
456
53%
24%
hGR
1
421
486
528
777
21%

Fig. 1

FIGURE 2


hRARα
1
88
153
198
462
DNA
LIGAND
hRARβ
1
81
146
191
448
97%
82%
hRARγ
1
90
155
200
454
97%
76%
hTRβ
1
102
169
232
456
62%
35%
hRXRα
1
135
200
239
462
61%
27%

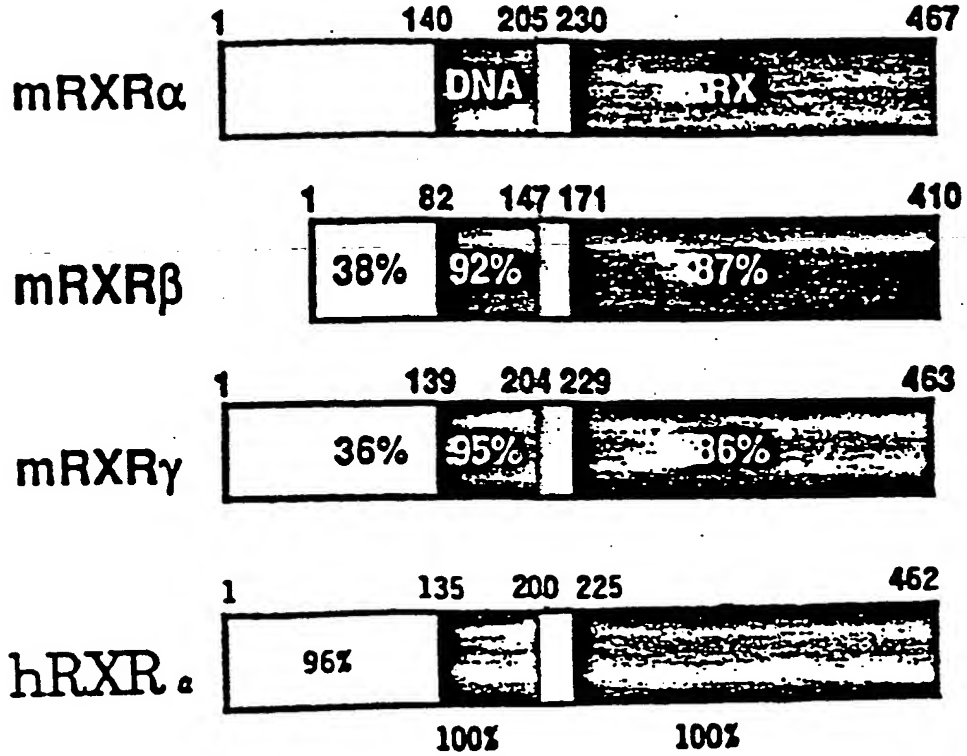
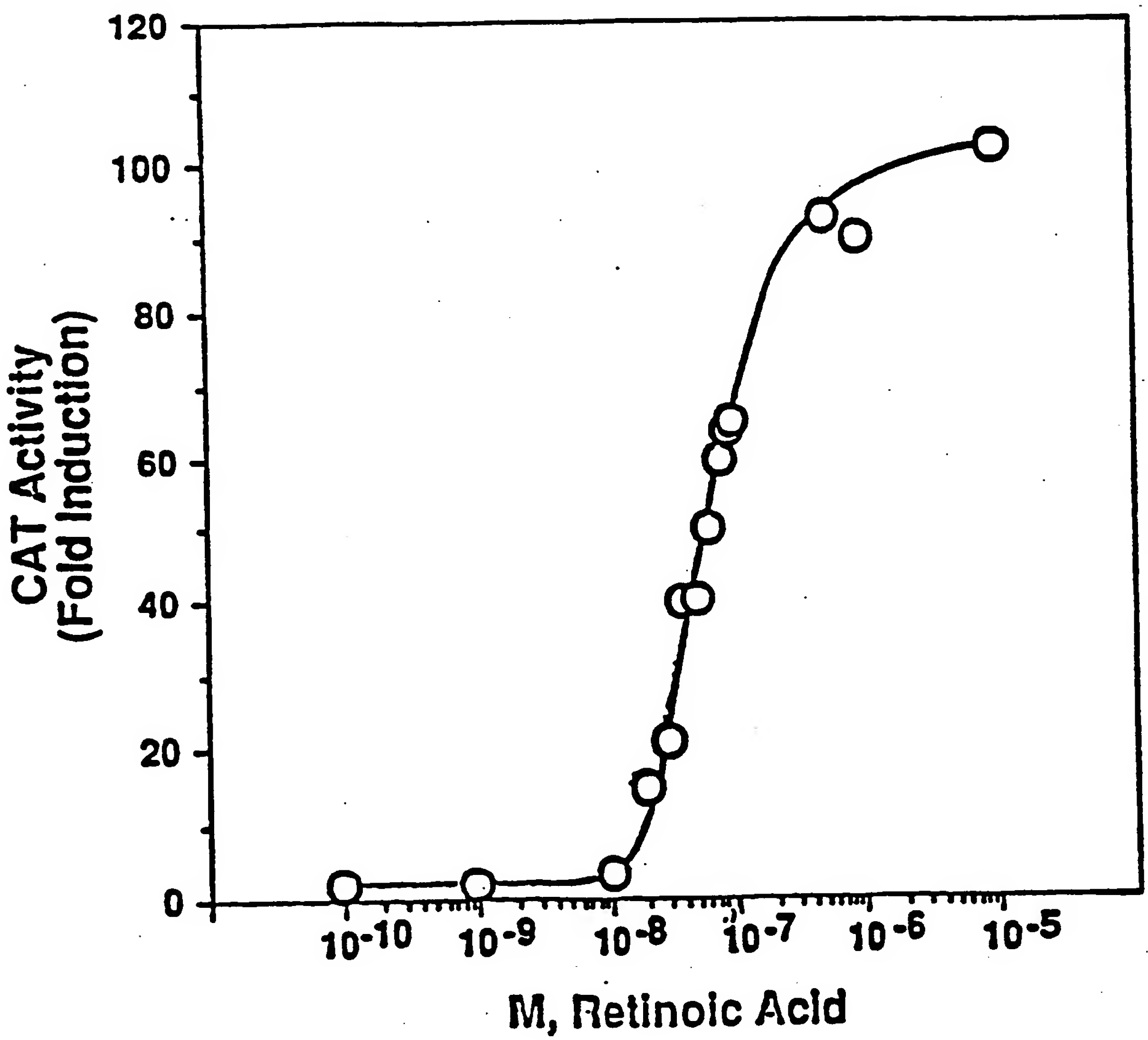

mRXRα
1
140
205
230
467
DNA
RX
mRXRβ
1
82
147
171
410
38%
92%
87%
mRXRγ
1
139
204
229
463
36%
95%
86%
hRXRα
1
135
200
225
462
96%
100%
100%

FIG. 3

FIGURE 4


120
100
80
60
40
20
0
CAT Activity
(Fold Induction)
10-10
10-9
10-8
10-7
10-6
10-5
M, Retinoic Acid

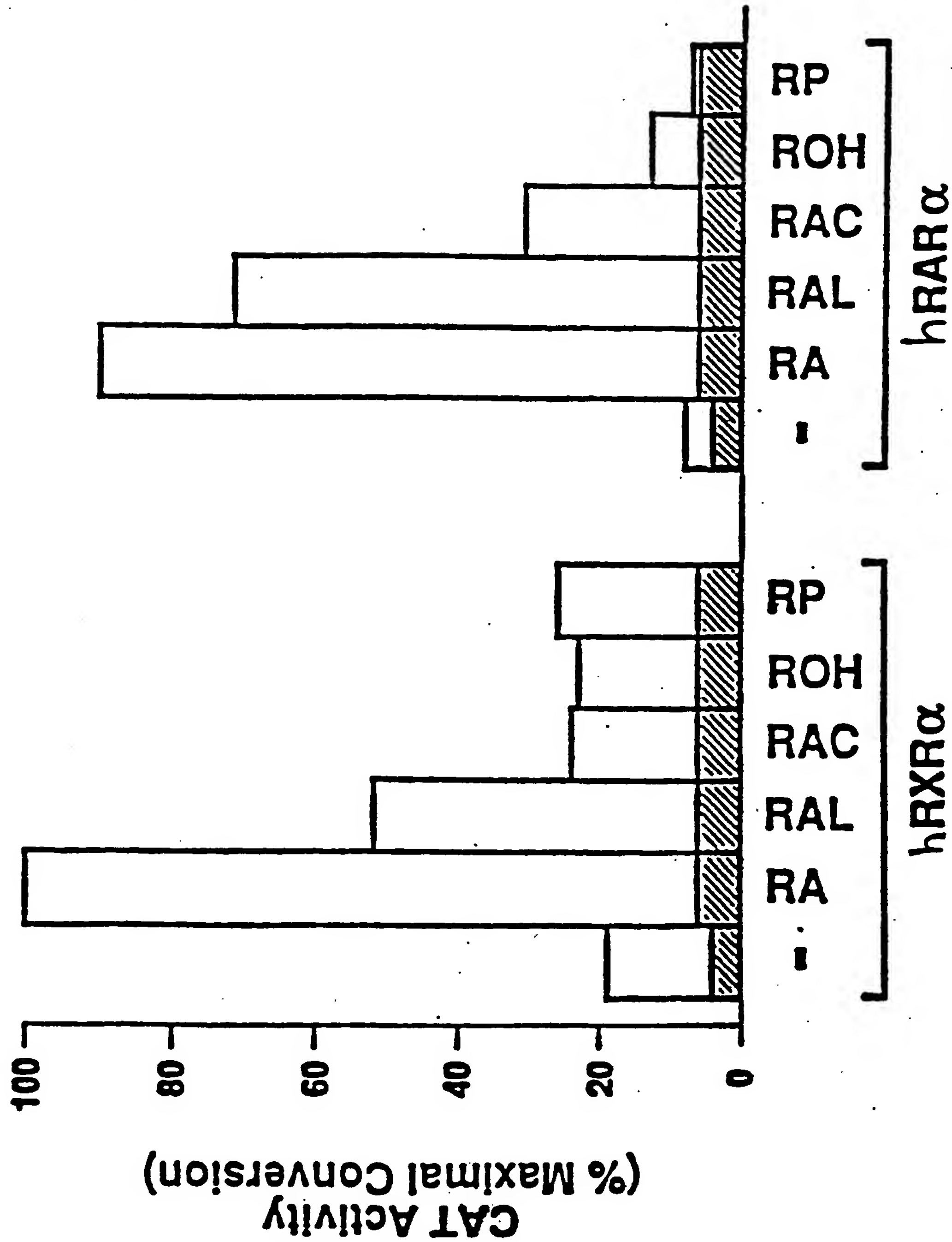

CAT Activity
(% Maximal Conversion)
0
20
40
60
80
100
-
RA
RAL
RAC
ROH
RP
hRXRα
hRARα

FIGURE 5

FIGURE 6

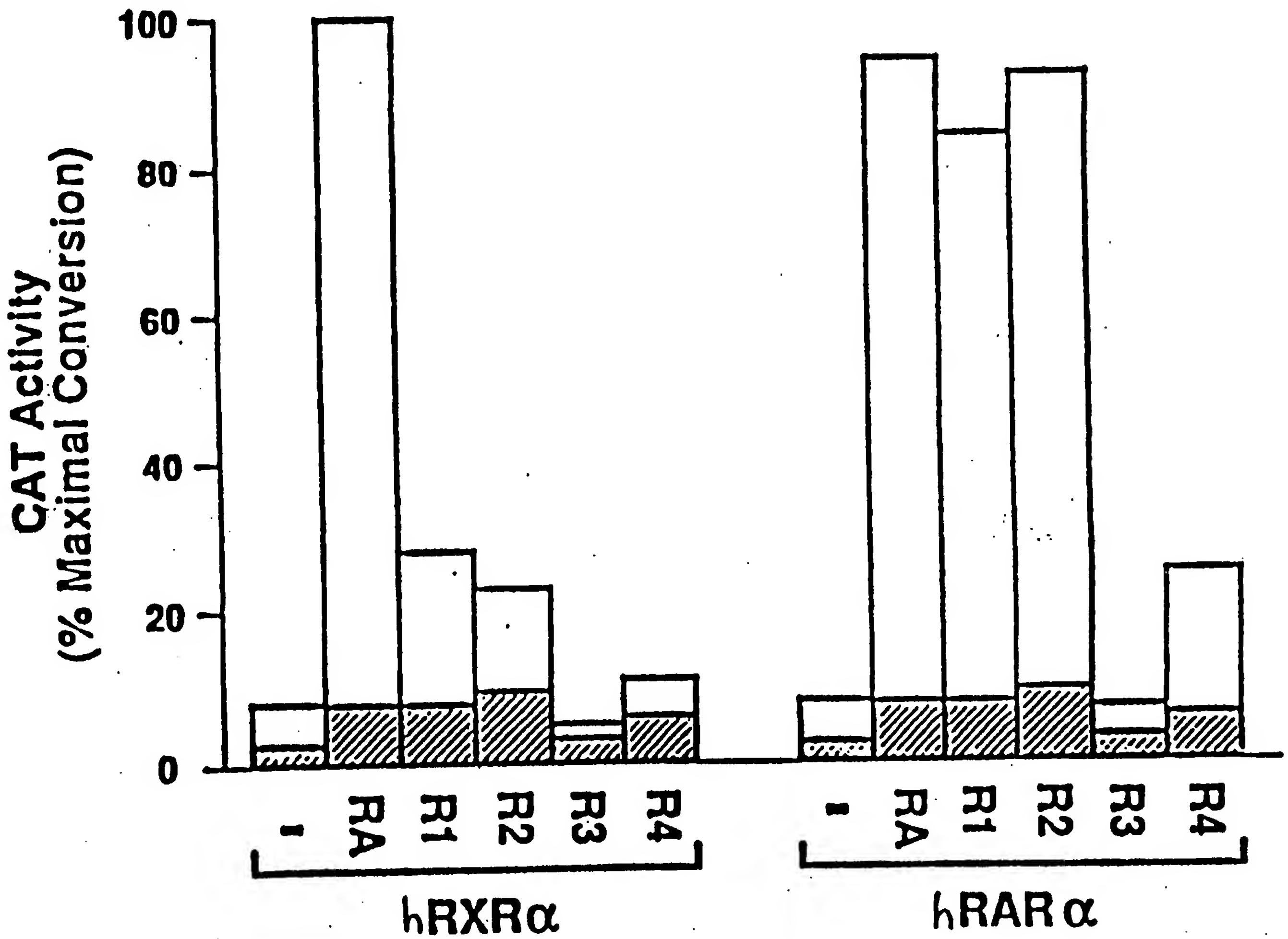
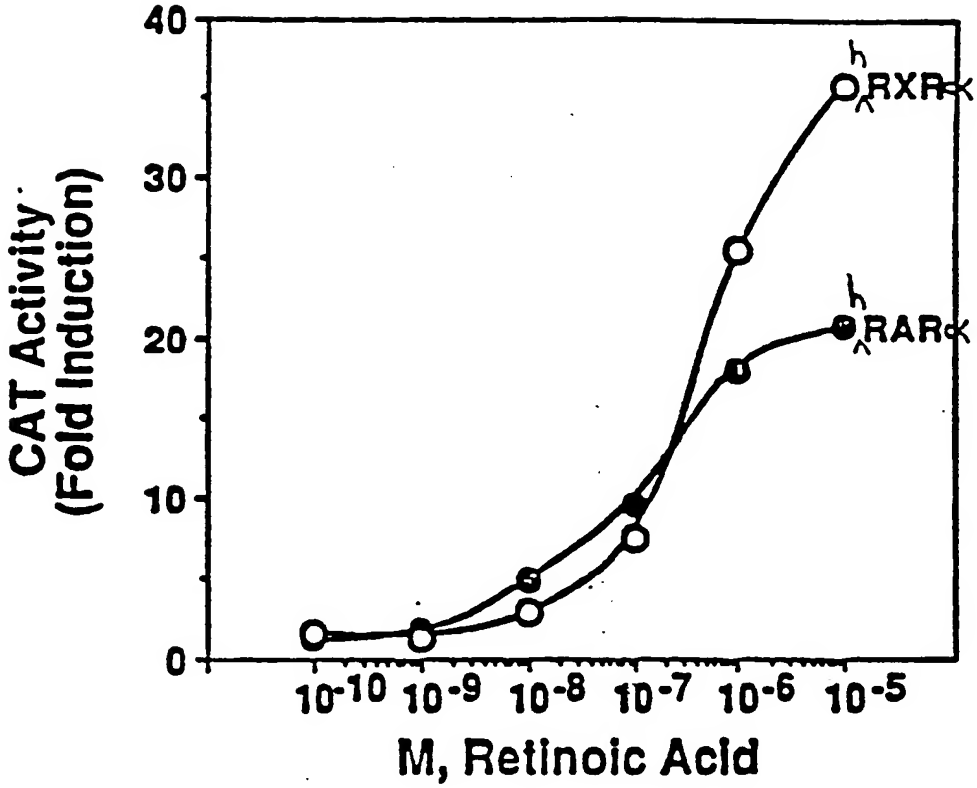

CAT Activity
(% Maximal Conversion)
100
80
60
40
20
0
-
RA
R1
R2
R3
R4
hRXRα
hRARα

FIGURE 7


40
30
20
10
0
CAT Activity
(Fold Induction)
hRXRα
hRARα
10-10 10-9 10-8 10-7 10-6 10-5
M, Retinoic Acid

a

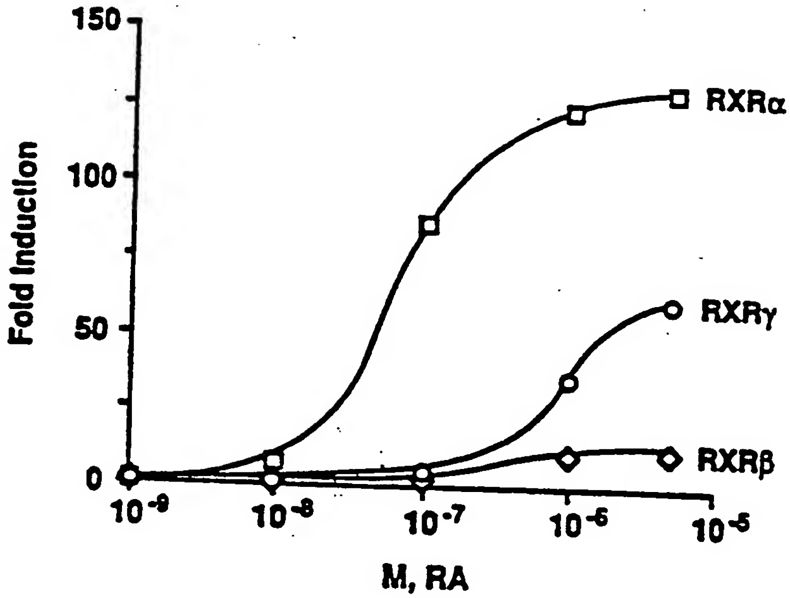

150
100
50
0
Fold Induction
RXRα
RXRγ
RXRβ
10^{-9}
10^{-8}
10^{-7}
10^{-6}
10^{-5}
M, RA

Fig. 8

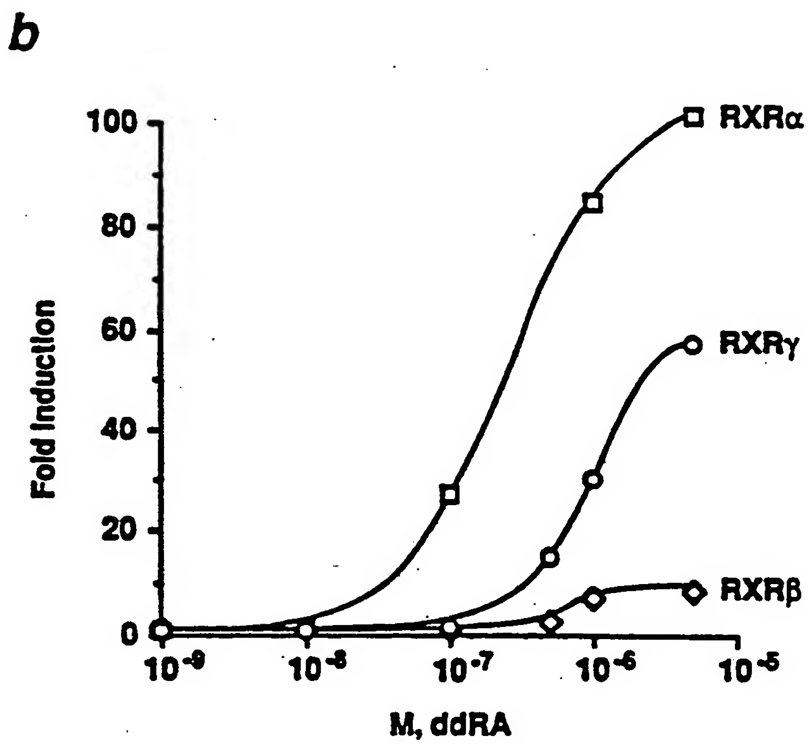

b
100
80
60
40
20
0
Fold Induction
10^{-9}
10^{-8}
10^{-7}
10^{-6}
10^{-5}
M, ddRA
RXRα
RXRγ
RXRβ

Fig. 9

INTERNATIONAL SEARCH REP RT

International Application No. PCT/US91/00390

I. CLASSIFICATION OF SUBJECT MATTER

According to International Patent Classification (IPC) or to both National Classification and IPC

I.P.C.(5): C07H 15/12; C07K 3/00; C12Q 1/68; C12N 15/00
U.S. Cl.: 536/27; 530/350; 435/6; 935/77.78

II. FIELDS SEARCHED

Minimum Documentation Searched

Classification System	Classification Symbols
U.S. Cl.	536/27; 530/350; 435/6; 935/77.78

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched

Gen Bank, EMBL

III. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	Nature, Vol. 337, issued 09 February 1989, Giguere et al, "Spatial and Temporal Expression of the Retinoic Acid Recepter in the Regenerating Amphibian Limb", pages 566-569, see especially Figure 1.	1-25
X	Nature, Vol. 330, issued 17 December 1987, Giguere et al, "Identification of a Receptor for the Morphogen Retinoic Acid", pages 624-629, see especially Figure 1.	1-25
X	Nature, Vol. 331, issued 07 January 1988, Giguere et al, "Identification of a New Class of Steroid Hormore Receptors", pages 91-94, see especially Figure 1.	1-25

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
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"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

IV. CERTIFICATION

Date of the Actual Completion of the International Search: 14 March 1991

Date of Mailing of this International Search Report: 03 MAY 1991

International Searching Authority: ISA/US

Signature of Authorized Officer: Mindy B. Fleisher

Form PCT/ISA/210 (second sheet)